TAMPEREEN TEKNILLINEN YLIOPISTO
TAMPERE UNIVERSITY OF TECHNOLOGY

HENRIIKKA YLIANNALA

SOLUBILITY OF ENZYME-TREATED CELLULOSE IN AQUEOUS ALKALINE SOLVENT

Master of Science thesis

Examiners: Professor Jurkka Kuusipalo and Project Researcher Taina Kamppuri
Examiners and topic approved by the Faculty Council of the Faculty of Engineering Sciences
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ABSTRACT

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Purpose of this thesis was to study the dissolution of enzyme-treated cellulose in aqueous alkaline solution. The first objective was to study the effect of zinc oxide on the solubility of the cellulose to aqueous NaOH solution. The second objective was to investigate the effect of cellulose and NaOH concentration on the solubility of cellulose with constant AGU:NaOH molar ratio. The third objective was to study the solubility of the cellulose at constant concentration by changing the NaOH:ZnO molar ratio and raising the concentration of NaOH.

In these experiments two different enzyme-treated cellulose samples were used. In the first two experiments the used pulp was mechanically shredded and enzyme-treated at TUT. For the third experiment the enzyme treated pulp was provided by VTT. The solubility was determined by freeze-thaw method, in which the samples were frozen at the desired temperature for at least 18 hours. The first two experiments were frozen at -40 °C, and the third at -15 °C. In addition, the solubility was analyzed at 20 °C (for the first two tests) and 15 °C (third experiment). After thawing the solubility of the samples were determined.

The effect of ZnO on solubility was clear. Adding ZnO increased the solubility of cellulose to aqueous NaOH. When the AGU:NaOH ratio was kept constant, the best solubility was obtained with the cellulose concentration between 5-6 wt%. In addition, cellulose was soluble at higher temperature when the cellulose concentration was slightly lower (4 wt%) and NaOH concentration was between 1.3-2.4 mol. However the effect of ZnO was not clearly seen and additional studies are needed to study this further. For example this can be achieved by increasing the concentration of the cellulose and the test temperature.

TIIVISTELMÄ

HENRIIKKA YLIANNALA: Entsyymikäsitellyn selluloosan liukoisuus alkaliseen vesiliuokseen
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Avainsanat: Entsyymikäsitelty selluloosa, alkalinen liuos, liukoisuus, ZnO

Tämän työn tarkoituksena oli tutkia entsyymikäsitellyn selluloosan liukenemista alkaliseen vesiliuokseen. Ensimmäinen tutkimuksen kohde oli sinkin vaikutus selluloosan liukoisuuteen natriumhydroksidin vesiliuoksessa. Toinen tutkimuksen kohde oli selluloosan liukoisuus, kun AGU:n ja NaOH:n suhde pidettiin vakiona. Kolmantena tutkimuksen kohteena oli selluloosan liukoisuus vakiokonsentraatiolla, kun muuttujina olivat NaOH:n ja ZnO:n moolisuhde ja NaOH:n konsentraatio.

Kokeissa käytettiin kahta eri entsyymikäsiteltyä sellua. Kahdessa ensimmäisessä kokeessa käytettiin TTY:llä mekaanisesti ja entsyymeillä käsiteltyä sellua. Kolmanteen kokeeseen käytettiin VTT:llä entsyymikäsiteltyä sellua. Liukoisuus kokeet tehtiin jäädytyssulatusmenetelmällä, jossa koekappaleet jäädytettiin halutussa lämpötilassa vähintään 18 tuntia. Kahdessa ensimmäisessä kokeessa jäädytyslämpötila oli -40 °C ja kolmannessa -15 °C. Lisäksi liuotuskokeita tehtiin 20 °C (kaksi ensimmäistä koetta) ja 15 °C (kolmas koe). Sulatuksen jälkeen näytteille tehtiin liukoisuusmääritys.

Sinkin vaikutus liukoisuuteen oli selvä. Lisäämällä ZnO:a selluloosan liukoisuus NaOH:n vesiliuokseen paranee huomattavasti. Kun AGU:n ja NaOH:n suhde on vakio, pystyttiin saamaan alue, jossa selluloosa parhaiten liukenee ja liuoksen laatu on hyvä. NaOH:n ja ZnO:n moolisuhdetta muuttamalla ja NaOH:n konsentraatiota nostamalla saatiin selluloosa lähes kokonaan liukenemaan huomattavasti korkeammalla lämpötilalla verrattuna aikaisempaan kokeeseen. Hyvä liukoisuus (yli 95%) saatiin usealla eri NaOH ja ZnO konsentraatiolla, jolloin lisätutkimuksia tarvitaan erottaan liukoisuuspisteitä. Tämä voidaan saavuttaa vaihtelemalla selluloosan konsentraatiota ja koelämpötilaa.

PREFACE

This thesis was written at Tampere University of Technology, the department of Materials Science as part of my Master's degree of Material Science.

First I would like thank Professor Emeritus Pertti Nousiainen for offering me the opportunity to write this thesis. My special thanks go to Taina Kamppuri and Marianna Vehviläinen for instructions, advices and all the help I had. For all the help in laboratory I would like to thank Maija Järventausta. I also would like to thank Ali Harlin and the people at VTT for their involvement in this thesis and providing raw materials. Finally I would like thank Professor Jurkka Kuusipalo and Taina for agreeing to inspect my thesis.

Last I would like thank my employer at that time for allowing me to have the time to write this thesis. My final thanks go to my Arnold for all the support and help had for writing this thesis.

Tampere, 25.5.2016

Henriikka Yliannala

CONTENTS

LIST OF SYMBOLS AND ABBREVIATIONS

AGU	D-anhydroglucose unit
AmimCL	i-allyl-3-methylimidazolium chloride
aq.	aqueous solution
BmimCL	1-butyl-3-methylimidazolium chloride
DMSO	dimethylsulfoxide
DNS	dinitrosalisylic acid
DP	degree of polymerization
EmimAc	1-ethyl-3-methylimidazolium acetate
e.g.	Latin exemli gratia, for exsample
et al.	Latin et alii or et alieae, and others
IL	ionic liquid
LiOH	lithium hydroxide
LCST	lower critical solution temperature
NaOH	sodium hydroxide
NH_3	ammonia
NMMO	N-methylmorpholine-N-oxide
P	primary wall
S	secondary wall
T	tertiary layer
TUT	Tampere University of Technology
UCST	upper critical solution temperature
VTT	Technical Research Centre of Finland
ZnO	zinc oxide
C_a	alkali concentration
C^*	critical overlap concentration
$C_{cell\ max}$	maximum cellulose concentration
E	eutectic mixture
ΔG	change of free energy
ΔH	change of enthalpy
T	temperature
Q	region in Na-cellulose phase diagram, where cellulose is soluble
S_a	solubility in aqueous alkaline solution
ΔS	change of entropy
wt%	percentage by mass

1. INTRODUCTION

As the population and standard of living improves the demands for fibers for textiles increases. As cultivation of cotton would take area from food productions and oil based raw material for man-made fibers will decrease, cellulosic fibers could answer for this demand. Today viscose process is one way to produce cellulosic fibers. However this process is has occupational health and environmental issues. Therefore investigating new methods to produced cellulosic fibers would answer demands of textile industry in future.

The dissolution of enzyme-treated pulp in to aqueous sodium hydroxide (NaOH) and zinc oxide (ZnO) solution and regenerating solution into fibers has been studied (Vehviläinen, et al., 2015). In the dissolution of cellulose the function of NaOH is to breakdown the hydrogen bonds between cellulose chains. This mechanism was found in the late 1800s and has been extensively studied since the early 1900s. The dissolution of cellulose can be enhanced by different additives. These include zinc oxide, urea and thiourea. The aim of this thesis is to study solubility of enzyme treated cellulose in aqueous NaOH/ZnO solutions.

This thesis has two parts, literature review and experimental part. In the literature review there is an overview of cellulose, its structure, morphology and bonding mechanisms. Then the dissolution of cellulose in alkaline solution and the rheological properties are discussed. Finally the essential phase diagrams of NaOH and cellulose in different solvents are presented.

In the experimental part there were three aims. First the effect of ZnO in the dissolution of cellulose in NaOH solution was studied. Second the solubility of cellulose in alkaline solubility at constant AGU:NaOH molar ration was studied. Third the optimization of conditions at different NaOH:ZnO molar ratio and varying the concentration of NaOH was studied. The determination of solubility was done by freeze-thaw method. There were two different pulps used in experiments. First pulp was mechanically shredded and enzyme-treated at Tampere University of Technology and the other enzyme-treated pulp was provided by VTT Technical Research Centre of Finland.

2. LITERATURE REVIEW

2.1 Cellulose

2.1.1 Chemical structure

The molecular structure of cellulose is presented in Figure 1. The empirical formula of cellulose is $C_6H_{10}O_5$. Cellulose is composed of repeating D-anhydroglucose units (AGU) and it can be considered to be a linear syndiotactic homopolymer. AGUs are linked to each other by β-1,4-glucosidic bond between the first and fourth carbon of neighboring units. The hydroxyl groups located in second, third and sixth carbons enable the AGU units to undergo etherification and esterification reactions. The hydroxyl groups located at the ends of the cellulose chain demonstrate different behavior. When the hydroxyl is at carbon 1 in the ring the end group is reducing. The glucose end group where the hydroxyl group is in the fourth carbon has non-reducing behavior. The oxygen atoms located in the ring and between the rings are for the most part involved in intra- and intermolecular interactions. (Klemm, et al., 1998; Krässig, 1993)

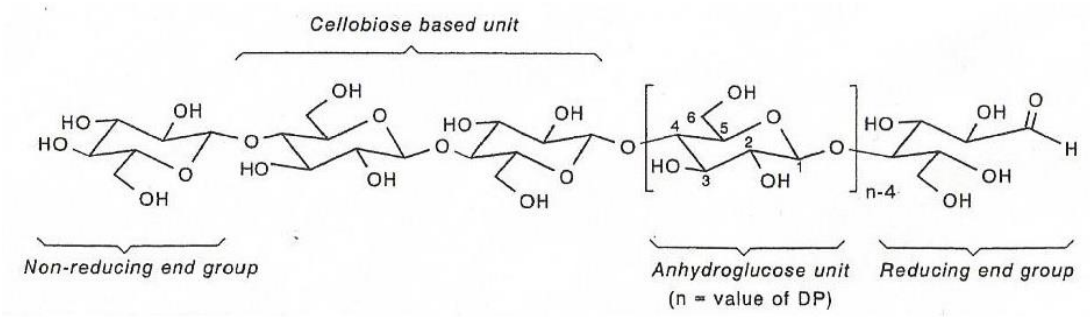

Figure 1 *Molecular structure of cellulose. (Klemm, et al., 1998)*

2.1.2 Crystal structure

Cellulose has four main polymorphs named cellulose I, II, III and IV. Figure 2 presents the know pathways to change crystalline structure of cellulose. Cellulose I is found in native cellulose (e.g. cotton, ramie). They have similar X-ray diffraction pattern and therefore are classified as cellulose I. Cellulose I consists of two crystalline modifications (I_α and I_β). Cellulose produced by primitive organisms and it is believed to have the I_α modification dominating whereas cellulose obtained from the higher plants have the I_β modification dominating. Cellulose I_α is considered to be metastable

and more reactive than I_β. Cellulose I_α can be transformed to thermodynamically more stable I_β by annealing in various media. Both of them have the same conformation of atom skeleton, but the hydrogen bonding pattern is different. Since native cellulose has these two polymorphs and they have different stabilities, this could lead to the I_α to be the site of initial reaction in a microfibril. (Klemm, et al., 1998; Krässig, 1993; O'Sullivan, 1997)

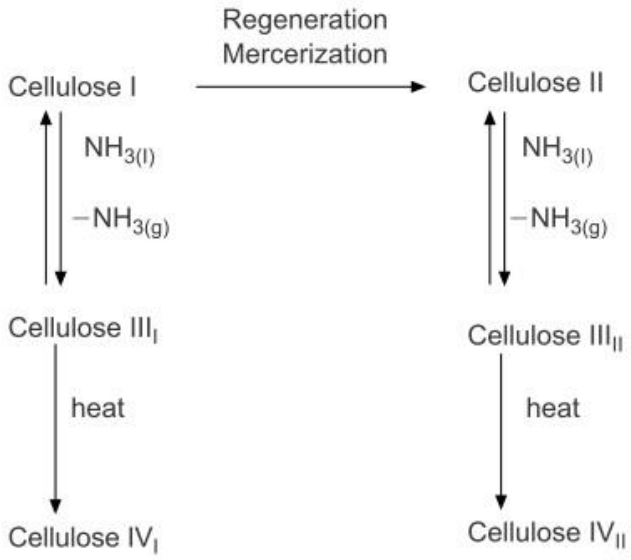

Figure 2 *Conversion of the different polymorphs of cellulose. (O'Sullivan, 1997)*

Cellulose II is obtained from cellulose I by two processes: regeneration or mercerization. In regeneration cellulose I is dissolved in suitable solvent and precipitated by anti-solvent. In mercerization the native fibers are swollen in concentrated sodium hydroxide and washed to remove the swelling agent. In mercerization process the fibrous structure of the cellulose is maintained and mainly no solubilization occurs, though some shorter chains and hemicellulose do dissolve. Regeneration produces higher level conversion of cellulose I to cellulose II. The modification of cellulose I to cellulose II would even out the difference in the structural characteristics of the original cellulose I. Transformation from cellulose I to cellulose II is considered to be irreversible. Cellulose II seemed to have more complicated hydrogen bonding system than cellulose I. Therefore cellulose II has higher intermolecular crosslinking density. (Klemm, et al., 1998; Krässig, 1993; O'Sullivan, 1997)

Cellulose III is produced by treating cellulose with liquid ammonia or certain amines at -30 °C and evaporating excess ammonia. Two forms, cellulose III_I and III_{II}, are obtained depending on the form of starting cellulose: cellulose I and cellulose II, respectively. The polarity of the cellulose III chain resembles the polarity of the starting material. Conversion from cellulose I to cellulose IIII involves decrystallization and fragmentation of the cellulose crystals. Partial recrystallization happens during

conversion back to cellulose I, but the fragmentation of the crystal is irreversible. (Klemm, et al., 1998; Krässig, 1993; O'Sullivan, 1997)

Cellulose IV is obtained from Cellulose III at elevated temperature in glycerol. Cellulose IV has two forms IV_I and IV_{II} obtained from cellulose III_I and III_{II} respectively, where the cellulose IV_{II} is best to use regenerated cellulose. The conversion from cellulose III to cellulose IV is in most cases only partial. (O'Sullivan, 1997)

2.1.3 Molecular interactions

The ultrastructure of cellulose is mostly due to the presence of covalent bonds, hydrogen bond, van der Waals forces and hydrophobic forces. This network enables the resistance to deconstruction by solvent or mechanical forces. (Gross & Chu, 2010)

Cellulose is polar molecule with several hydroxyl groups. These hydroxyl groups can interact with each other to form secondary valence bonds called hydrogen bonds. The strength of the hydrogen bonds is about 100 times stronger than the van der Waals forces but less than one-tenth of the O-H covalent bond strength. Hydrogen bonds can be formed by either interaction between hydroxyls groups in the same molecule or interaction between hydroxyl groups in the neighboring cellulose molecules. (Krässig, 1993; Lindman, et al., 2010)

Figure 3 shows the intra- and interchain hydrogen patterns for cellulose I and II. Cellulose I has intrachain bonds at O6-H-O3 and O2-H-O6 and an interchain hydrogen bond at O6-O3. Cellulose II has an intrachain hydrogen bond at O3-H-O5 and interchain hydrogen bonds at O6-H-O2 for corner chains and O6-H-O3 for center chains. (O'Sullivan, 1997)

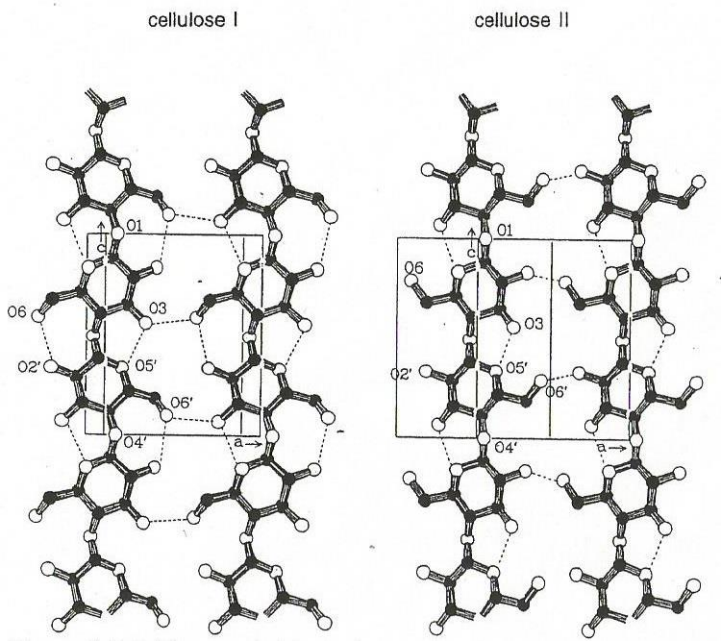

Figure 3 *Hydrogen bonding pattern for cellulose I and cellulose II. (Klemm, et al., 1998)*

In both polymorph of cellulose I the molecule chains are arranged in parallel to form flat sheets, which are stacked on top of each other to from full three-dimensional crystal structure. In intersheet contacts the C-H-O contacts and van der Waals interactions connects residues on neighboring sheets. The strength and robustness of cellulose is caused by this interaction network. In intersheets interaction the C-H-O pseudo hydrogen bond and van der Waals are expected to be involved. Among the three type of bonds intersheet interactions seemed to be most stable. The number of intersheet bonds differs between I_α and I_β microfibrils. In solvent exposure on the interaction network of cellulose the reduction of strength is only observed at outer chains, whereas the chains in the layers further inward are not affected. This is explained by the distance to microfibril surface. There are significant reductions of strength in solvent-exposed chains, since the intra- and interchain hydrogen bonds can form hydrogen bonds with water molecules. (Gross & Chu, 2010)

Cellulose has an anisotropic structure. All three hydroxyl groups are on the equatorial positions of the glycopyranose ring making this direction hydrophilic. The hydrogen atoms of C-H bonds are located on the axial position of the ring making this direction hydrophobic. The chain configurational entropy doesn't seem to favor either crystal from or the solution form. The hydrophobic interaction and hydrogen bonding seemed to favor crystal form. The conformation of the β-(1→4) linkage and the hydrophobic characters lead to the planar topology of cellulose chains. This topology is partially reason for the favorable energy contribution to either crystal form or solution. (Bergenstråhle, et al., 2010; Yamane, et al., 2006)

Cellulose II has an intersheet interaction between O2-H (corner chain) – O2 (center chain). Cellulose I does not have this kind of interaction. The splitting and reformation of the intra- and intermolecular hydrogen bonds determine the transformation from cellulose I to II. The polarity of the chains differs in cellulose I and II, which also influences on the differences of the hydrogen bonding patterns. Cellulose I has parallel arrangement and cellulose II antiparallel arrangement of the chains. The relative shift of the center and corner chains along the fiber axis further affects the intersheet interactions. (O'Sullivan, 1997)

2.1.4 Morphological structure

Microfibrils consist of cellulose molecules and are the basic unit of the morphological structure of cellulose. This structure is presented in Figure 4. Microfibrils are combined into a larger morphological entity with diameters of 10-50 nm depending on the cellulose origin. The length of micro-fibrils can be micrometers. Macrofibrils are composed of microfibrils and the diameters range in micrometers. (Klemm, et al., 1998)

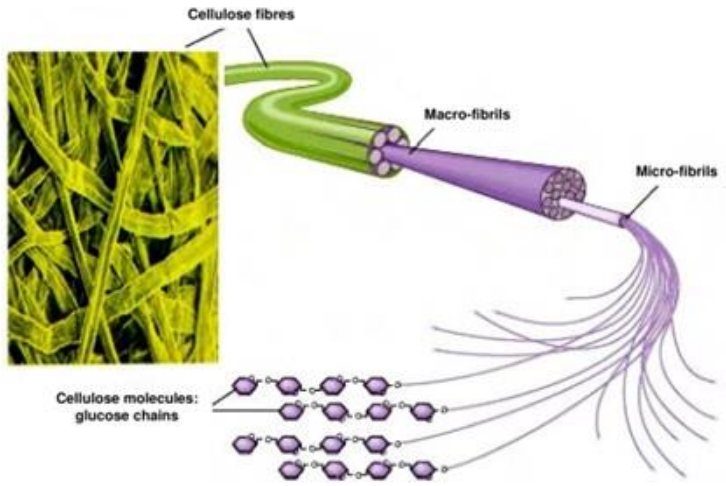

Figure 4 *The structure of cellulose fibers. (Egal, 2006)*

Micro- and macrofibrils are construction units for the cell-wall structure in cellulose fiber appearing in nature. In native cellulose the structure is organized in layers. Some generalities exist between cellulose of different origins. They usually have the same layers, but the alignment of the microfibrils differs. The supramolecular structure of cotton and spruce fiber are presented in Figure 5. The outer layers of fibers consist of primary wall (P) with diameter of 10 nm. Secondary wall (S) consists of two layers S_1 and S_2. The thickness of S_1 has different values e.g. 100 nm for cotton or 300 nm for spruce pulp fiber. The fibrils are in parallel alignment and densely packed in a flat helix. The S_1 layer can prevent the swelling of S_2 layer. The thickness of S_2 can be several µm and it contains most of the cellulose mass. The fibrils are aligned in a helix. The tertiary layer (T) is the closet to the lumen. This layer is thin and the fibrils are aligned in a flat helix. (Klemm, et al., 1998)

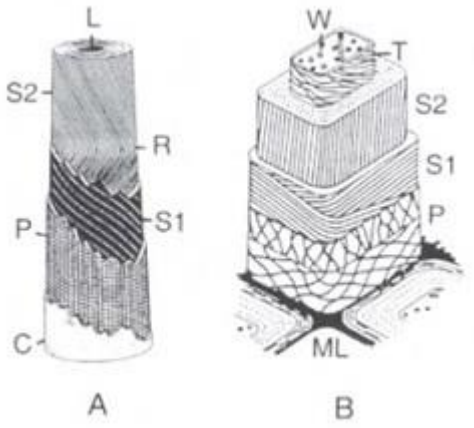

Figure 5 *Morphological architecture of cotton (left) and delignified spruce fiber (right). (Klemm, et al., 1998)*

2.2 Dissolution in alkaline aqueous solution

The dissolution is subject to the free energy of mixing. If the mixing is spontaneous the change of free energy has to be considered:

$$\Delta G = \Delta H - T\Delta S,$$

where ΔG is change of free energy, ΔH is the change of enthalpy, T is the kelvin temperature and ΔS is the change of entropy. If the free energy change is negative, the mixing process will happen spontaneously. If the free energy change is positive, phase separation will occur. If the molecular weight is high the entropic driving force contributing to dissolution is weak. (Medronho & Lindman, 2015; Zumdahl, 2005)

2.2.1 Cellulose

The dissolution of cellulose starts with swelling, where solvent molecules penetrate the structure. In cellulose fibers the swelling mostly appears as ballooning phenomenon in selected zones of the fiber. This phenomenon can be explained by the swelling of the secondary wall, which causes the primary wall to extend and burst. The primary wall can form collars, rings and spirals, when the swollen cellulose pushes through the primary wall. Cellulose presented inside of the secondary wall dissolves easily. When primary wall is presented the S_1-layer forms a membrane. If there is no primary wall the S_2-layer forms the membrane instead. The membrane of the balloon can burst when the osmotic pressure inside the balloon is greater than the breaking resistance of the membrane. Also the membrane can be mechanically broken by e.g. mixing in a mechanical mixer. If the wall structure of the fiber is at its original state, the mechanism of dissolution is similar for wood, cotton, other plant fibers and some cellulose derivatives. To have complete dissolution the supramolecular structure of cellulose

should be completely destroyed. (Budtova & Navard, 2015; Cuissinat & Navard, 2006; Medronho & Lindman, 2015)

The dissolution and stability of cellulose solution is highly influenced by the average degree of polymerization (DP) of cellulose. The DP value of cellulose depends on the on the origin and pretreatments of the cellulose. The DP of cellulose has found to be the dominating factor in dissolution rather than crystal form of the cellulose. Cellulose, with DP lower than 200, can be easily dissolved and cellulose with higher DP could only be partially dissolved. The leveling-off DP refers to the DP where the reduction of DP is rapidly leveled during the hydrolysis of cellulose. Depending on the origin of cellulose, each sample has a certain leveling-off DP, and cellulose with DP equal or under of this value can be dissolved in to aqueous NaOH solutions. Plant-derived cellulose has higher DP than the leveling-off DP. Therefore these cellulose fibers are only partially soluble. (Cuissinat & Navard, 2006; Egal, 2006; Isogai & Atalla, 1998; Klemm, et al., 1998; Le Moigne, et al., 2010; Vanreyren, 2010)

Pre-treating the cellulose has a role in speeding up the dissolution. This is also called activation and it increases the kinetics of dissolution. Before dissolution, the activation of cellulose increases the number of paths for the reagents into the cell structure, which brings the reagents closer to the cellulose chains. Before the dissolution in NaOH the activation can be done by mechanical, physical, chemical and enzymatic methods. Sisal pulp has been argued to dissolve better in certain solvent than unmodified cotton linter. This might be due to the smaller crystalline size and the macro-pores in the supramolecular structure, which eases the solvent penetration. However after mercerization of the cotton linter the dissolution improved. Mercerization of the linter decreased the crystallite size. (Budtova & Navard, 2015; Ramos, et al., 2011)

The crystal structure of cellulose has an effect on its dissolution. Cellulose II is more difficult to dissolve in an alkaline solution than cellulose I. This is due to the more complex hydrogen bonding network. The dissolution of cellulose II is easier than of cellulose III. Cellulose III was more stable due to the lower energy structure after evaporation of the ammonia. This indicates that the solubility of the cellulose can be enhanced by decreasing DP of cellulose. (Chen, et al., 2015; Lindman, et al., 2010)

In the three dimensional arrangement of cellulose molecules, the oxygen atoms are within the sheet and form hydrogen bonds with hydroxyl groups inside the sheet (see Figure 3). Therefore the structure of the sheet is hydrophobic. This makes the gap between the sheets difficult to penetrate by the solvent in untreated cellulose. The solvent that do penetrate is not strong enough to destroy the stacking of the sheets. When the intrachain hydrogen bonds are partly broken the solvent molecules can penetrate more easily into the widened gap between the sheets in the amorphous area. This could lead to the destruction of the remaining intrachain hydrogen bonds leading to

the destruction of the crystalline region and total dissolution. Figure 6 presents the mechanism of cellulose in aqueous alkali solution. (Kamide, et al., 1992)

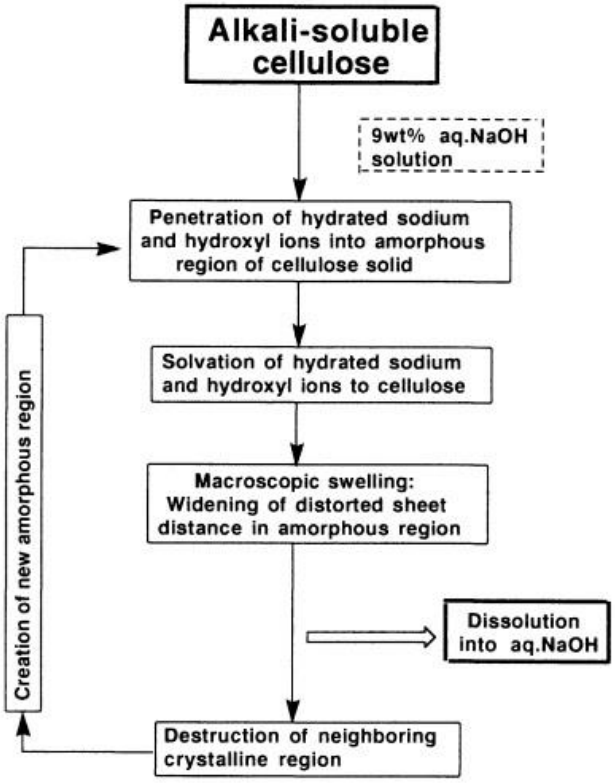

Figure 6 *The mechanism of cellulose in aqueous solutions. (Kamide, et al., 1992)*

Cellulose has a ribbon like-like conformation due to the equatorial β-(1→4)-linkage and never exists as a single chain. The hydroxyl groups in cellulose are equatorial and non-hydrogen bonding protons occupy all of the axial position. Therefore the sides of the cellulose chains are polar and have inclination to form hydrogen bonds. Also for the same reason the top and the bottom of the chain are hydrophobic. The chains stack together so that the hydrophobic phases are matched and hydrogen bonds are formed between chains. In water the conformation is adjusted so that the contact between hydro phobic parts and water is reduced. The conformational change enhances the dissolution of cellulose. (Lindman, et al., 2010; Matthews, et al., 2006)

2.2.2 Temperature

Decrystallization reaction is exothermic, favoring lower temperature. In non-isothermal process the decrease of cellulose crystallinity occurs with the decrease of temperature. An optimum alkali concentration and cooling temperature exist. Free water appears when cellulose/NaOH/water solution is kept below -20 °C for several hours. This water crystallizes and melts at 0 °C. The large amount of crystallized water changes the environment of the solution. As the temperature decreases more ice is produced and the

concentration of NaOH increases. This leads to increase of NaOH concentration in the immediate environment of cellulose, high adsorption of NaOH to cellulose and the change of solubility. This could be the reason why freezing step influences on the final properties of regenerated cellulose. (Egal, 2006; Wang & Deng, 2009)

The solvent for cellulose dissolution must overcome the low entropy gain. As the temperature lowers the network of NaOH hydrates become stronger. This might be due to the increase of strength of the hydrogen bonding. This network prevents the cellulose chains from interacting with each other and forming interchain hydrogen bonds. Raising the temperature weakens the hydrogen bonds and the network is destroyed. (Egal, et al., 2007; Medronho & Lindman, 2015; Zhang, et al., 2010)

The dissolution of cellulose in alkali solution can be described as entropy-driven process, when the solution is pre-cooled to low temperatures. The cellulose in solution state has lower enthalpy than in solid state. In low temperature the hydrogen-bond structure is created between cellulose macromolecules and the solvent. This leads to form of inclusion complex, which bring the cellulose into the solution. The enthalpic factors favoring dissolution are better at lower temperatures. Raising temperature the cellulose-cellulose interaction becomes more favorable than cellulose-NaOH interactions. (Budtova & Navard, 2015; Qi, et al., 2008)

2.2.3 Concentration of NaOH

The role of NaOH in dissolution of cellulose is to penetrate between and into the crystallites. Inside the crystallite structure NaOH destroys the inter- and intramolecular-hydrogen bonds of cellulose molecules. The limit for cellulose dissolution is NaOH/water is at least four NaOH molecules per one AGU-unit. In other words the weight ratio of cellulose/NaOH is one. Any higher concentrations cellulose does not dissolve. Since the range of NaOH concentration where cellulose dissolves is narrow (7-10%), the maximal amount of cellulose concentration is 10%. This limit is obtained by low DP cellulose and at higher DP cellulose insoluble part is presented. (Egal, et al., 2007; Zhang, et al., 2010)

With the NaOH concentration below 6-7% too much water exists in the solution and the amount of NaOH is too little to dissolve cellulose. Also the size of the hydrate might be too large to penetrate cellulose fibers. At higher concentration of NaOH (18-20%) and lower amount of water, the NaOH stays closer to cellulose and forms a Na/cellulose crystal. If the concentration of NaOH and the amount of water is between these limits the NaOH hydrate penetrates the cellulose fibers and binds to chains without forming Na/cellulose crystal. (Egal, et al., 2007; Zhang, et al., 2010)

Kuo & Hong (2005) showed that the optimal range for NaOH concentration was between 7.1 and 14.9 wt% when the cellulose concentration of the solution was under

5 wt%. The optimal concentration range of NaOH shrinks as the concentration of the cellulose increases. If the concentration of NaOH is decreased so that the number of Na^+-ions is low, the water brought by these ions to cleave the hydrogen bonds of the cellulose is insufficient. Poor dissolution process was caused by the insufficient swelling, which is caused by the insufficient number of the available Na^+-ions. Also too high concentration of Na^+-ions causes low level of the hydration of alkali ions, which leads to poor swelling of cellulose by insufficient capacity of Na^+ to cleave hydrogen bonding. Yamashiki et al (1988) showed that 9% NaOH was the optimal to dissolve cellulose. Figure 7 demonstrates the solubility of cellulose as function of NaOH and LiOH concentrations.

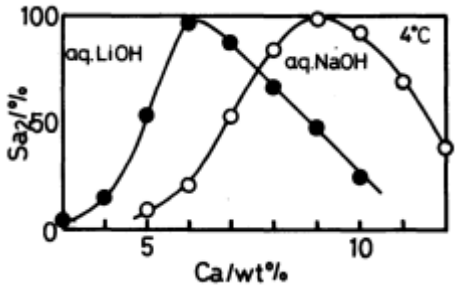

Figure 7 *Solubility of cellulose (S_a) at 4°C as function of alkali concentration (C_a): ○aqueous NaOH, ●aqueous LiOH. (Yamashiki, et al., 1988)*

2.2.4 Concentration of NaOH/ZnO

ZnO can be dissolved either in acid or strongly basic aqueous solutions. Dissolving solid state ZnO induces some reaction with OH^- to form zinc hydroxyl ions according to the following equilibrium equations: (Egal, 2006)

$$ZnO_{(8)} + H_2O + OH^- \rightleftharpoons Zn(OH)_3^-$$

$$ZnO_{(8)} + H_2O + 2OH^- \rightleftharpoons Zn(OH)_4^{2-}$$

When the ZnO is added to NaOH/water solution the described reactions will occur to dissolve ZnO crystals. To dissolve ZnO need not only Na^+/OH^- ions but also large quantity of water to be dissolved. It is assumed that 25 water molecules are needed for dissolving one molecule of ZnO and the limit of ZnO dissolution is at NaOH/ZnO molar ration of 10. (Egal, 2006)

When ZnO reacts to NaOH at pH=6-14, $ZnOH^+$-, $Zn(OH)^-$- and $Zn(OH)_4^{2-}$-ions and at higher concentrations (pH=>12) $NaZn(OH)_3$ and $Na_2Zn(OH)^4$ are produced. The solubility of $NaZn(OH)^3$ increases with increase of alkali concentration. (Chen, et al., 2012)

Excessive amount of ZnO can decrease the contribution of NaOH to cellulose dissolution. In the alkali solutions the zincate exists primary as $Zn(OH)_4^{2-}$-anions. Cellulose has been suggested to form stronger hydrogen bonds with $Zn(OH)_4^{2-}$ than with NaOH hydrate. It is easier to form new hydrogen bonds between $Zn(OH)_4^{2-}$ and cellulose due to the relatively slow motion compared with NaOH. It has also been suggested that $Zn(OH)_4^{2-}$-anions charges up cellulose by forming cellulose-zincate charged complex. (Kihlman, et al., 2013; Yang, et al., 2010)

Increasing the molar ratio of ZnO and NaOH at constant temperature the maximum solubility of cellulose can be increased significantly. It have been found that the solubility of enzyme-treated cellulose was the highest in ZnO/NaOH containing 1.3 wt% of ZnO and 5.5-6.5 wt% of NaOH and thermally the most stable solution was the one containing 1.3 wt% of ZnO and 6.5 wt% of NaOH. (Vehviläinen, et al., 2015)

2.3 Rheological properties

At very low concentration of cellulose the solution has Newtonian behavior. By increasing the concentration of cellulose the solution starts to have typical pseudoplastic properties. Reason for this is the aggregation of molecular chains. The linearity of the cellulose molecules allows them to be aligning in the flow direction that is the reason for the shear thinning behavior. Dilute solution (0.80-1.2%) flow like Newtonian fluids. Semi-dilute solution (below 5%) shows a Newtonian plateau and a shear-rate range. Above 5% the Newtonian plateau disappears. (Lue & Zhang, 2009; Roy, et al., 2003; Vehviläinen, et al., 2015)

When cellulose concentration increases, the viscosity of the solution increases. This is due to the increase of hydrogen bonding with molecule chains and distortion in the velocity pattern of the liquid by hydrated molecules of the solute. The intermolecular distance is inversely proportional to viscosity due to the changing temperature. Also this distance affects the viscosity. The shear thinning behavior is caused by disentanglement of polymer coils in solution or by an increased orientation of the polymer coils in flow direction. In addition the increased cellulose concentration and increased temperature increases the viscosity of cellulose solution. Reason for this could be gelation, which is accelerated at higher temperature. On the other hand viscosity of dilute cellulose solution, where gelation phenomenon doesn't exist, decreases when the temperature increases. (Roy, et al., 2003; Vehviläinen, et al., 2015; Zhang, et al., 2011)

If the concentration of the cellulose is too high, weak gelation network starts to form. The Newtonian behavior is mostly seen in the solutions that have concentration below the critical overlap concentration (C*). In this behavior the moving sphere- or cube-like inclusion complexes are separated from each other. In the concentration above C* the molecules starts to overlap with each other. Further increase in the concentration to regimen higher than the entanglement concentration, where the chains might become

entangled and form three-dimensional network. A sing of gelation is, with time, the difference between the elastic and loss moduli. When the difference becomes smaller the gelation takes place. The gelation of semi-dilute cellulose/NaOH solution is faster at higher temperatures. (Lue & Zhang, 2009; Roy, et al., 2003)

As the temperature increases, the role of hydrophobic interactions increases. Cellulose agglomerates become more compact because of the inter-chain hydrophobic interaction with the increase of temperature. Because of inter-agglomerate interactions, new compact agglomerate is formed with hydrodynamic volume smaller than the additive sum of volumes of the initial agglomerates. (Roy, et al., 2003)

2.4 Phase Diagrams

Phase diagram is described as a two-dimensional map showing which phases are able to exist in an equilibrium state under given conditions. Phase diagrams can be either binary or ternary systems. The variables are temperature, pressure and composition. Usually phase diagrams are drawn two-dimensional, either temperature-composition diagram at constant pressure or pressure-composition diagram at constant temperature. (DeVoe, 2015)

Two polymers, which are mutually dissolved, are found to be phase-separated at higher temperatures rather than lower temperature. This is called lower critical solution temperature (LCST). Low entropy of mixing cases high-molecular-weight polymer blends to show LCST phenomenon. Upper critical solution temperature can be seen if the chains are short. Figure 8 shows phase diagrams of polymer solution on temperature-composition plane. Figure 8a has the critical temperature at highest point of the curve referred as upper critical solution temperature (UCST). Figure 8b has the critical temperature at the lowest point of the curve referred as lower critical solution temperature (LCST). (Sperling, 2006; Teraoka, 2002)

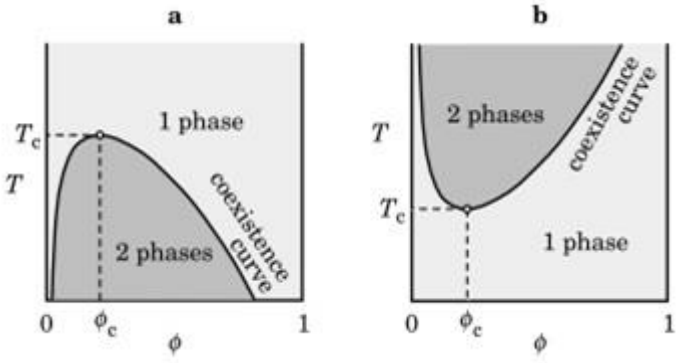

Figure 8 *Phase diagrams of a) UCST-type phase diagrams and b) LCST-type phase diagrams. (Teraoka, 2002)*

Temperature, pressure and concentration control the phase separation and dissolution. A ternary phase diagram has three components. Usually ternary phase diagrams are drawn at constant temperature and pressure. Figure 9 presents the basic of ternary phase diagrams. The components A, B and C are represented in each vertex of the equilateral triangle. Figure 8a presents the concept of deterring mole fractions. Mole fraction z_a is determined by measuring the distance to the point from the side that is opposite the vertex for A and express the distance as fraction of height of triangle. Same procedure is applied to determine z_b and z_c. Equally-spaced lines within the triangle parallel to sides can be drawn to aid the conversion between the position of a point and the overall composition. This is illustrated in figure 9b. (DeVoe, 2015)

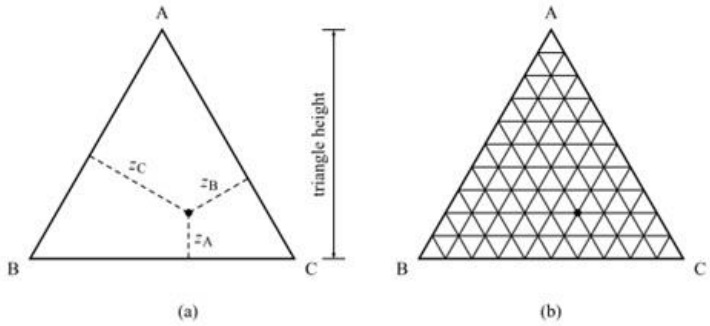

Figure 9 *Ternary phase diagrams. (DeVoe, 2015)*

In main polymer research one of the variables is held constant while other two are systematically varied. Simultaneous variation of all three variables generates more complex three-dimensional figure. A typical phase diagram of polymer-polymer miscibility analysis is presented in Figure 10. The solid line is called bimodal and dashed line the spinodal. Phase separation will occur when the temperature is raised through the bimodal line. As the pressure increases miscibility increases. Removing solvent causes the solution to become less miscible. As going from point A to point B mixture undergoes phase separation. As the monomer polymerizes to polymer II phase separation occurs during polymerization (point A to point B). (Sperling, 2006)

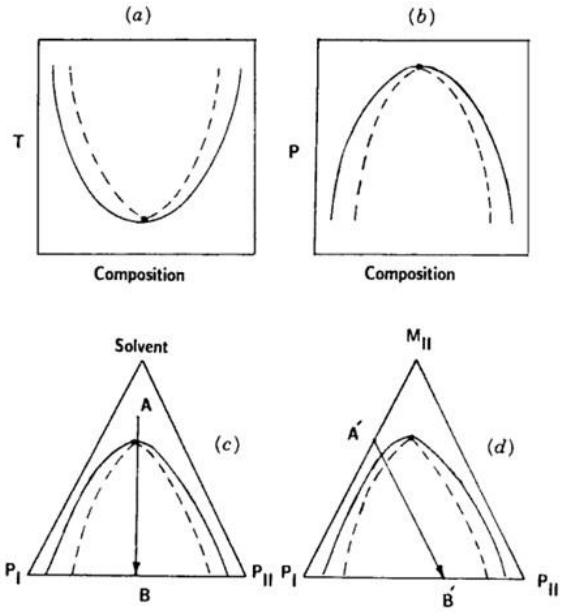

Figure 10(*a) LCST behavior of a typical polymer blend.(b) Corresponding behavior as a function of pressure. (c) Phase diagram of a solvent and two polymers P_I and P_{II}. (d) Mutual solution of polymer P_I and monomer M_{II}. (Sperling, 2006)*

2.4.1 NaOH phase diagrams

NaOH is a strong alkali and highly hygroscopic. NaOH absorbs moisture from the air and dissolves in it. Therefore NaOH occurs as hydrates. The solubility of NaOH to water increases with temperature.

The number of water molecules in the formation of stable sodium hydrates depends on the NaOH concentration and on the solution temperature (Figure 11). Eight hydrates of sodium hydroxide have been identified: (Egal, 2006)

- monohydrate $NaOH \cdot H_2O$
- dihydrate $NaOH \cdot 2H_2H$
- trihydrate $9NaOH \cdot 28H_2O$
- 3.5 hydrate $NaOH \cdot 3.5H_2O$
- two tetrahydrates NaOH, $4H_2O$ α and NaOH, $4H_2O$ β (where α and β are crystalline forms of NaOH)
- pentahydrate $NaOH \cdot 5H_2O$
- heptahydrate $NaOH \cdot 7H_2O$

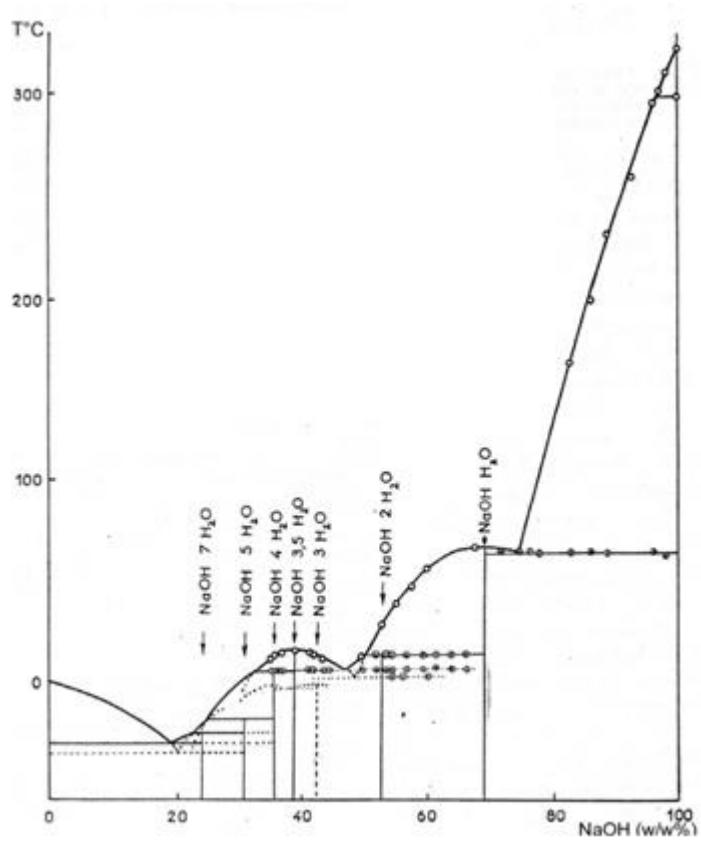

Figure 11 *Binary phase diagram of NaOH/water system. Y-axis corresponds temperature and X-axis corresponds NaOH weight percentages. (Egal, 2006)*

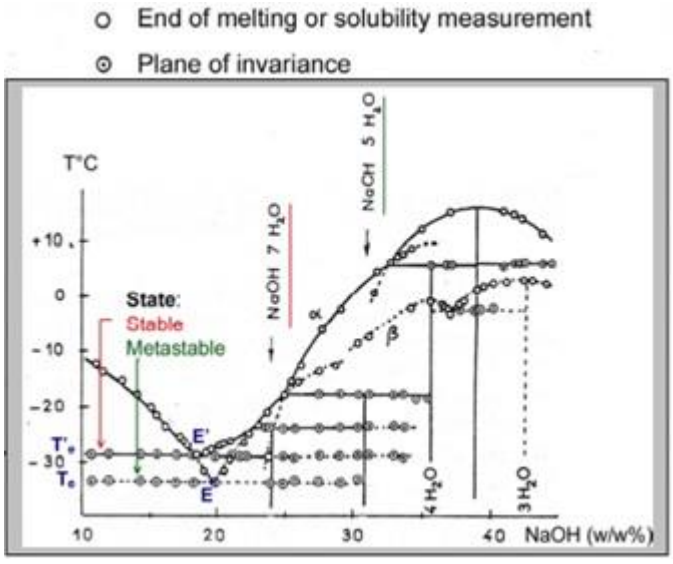

Figure 12 *Stable and metastable NaOH hydrates in crystallized state. Y-axis corresponds temperature and X-axis corresponds NaOH weight percentages. (Egal, 2006)*

Figure 12 points E and E´ that correspond the eutectic mixtures (the melting point of the mixture is lower than any of the meting points of the components) formed by ice and NaOH. In this eutectic mixture there are two possible hydrates: NaOH, $7H_2O$ and NaOH, $5H_2O$. The stable NaOH, $7H_2O$ hydrate is obtained after rapid temperature drop

followed by isothermal step at higher temperature. The metastable NaOH, 5H2O is obtained by slowly decreasing temperature. In the range of 0-31% NaOH the mixture of sodium hydroxide and water is a simple solution. NaOH and water are miscible in liquid state and immiscible in solid state. (Egal, 2006)

It is known that ZnO does not dissolve in water but the solubility can be improved by raising or lowering the pH of the solution. While dissolving in water the following ions presented $Zn^{2+}(aq)$, $Zn(OH)^{+}$ (aq), $Zn(OH)^{2}(aq)$, $Zn(OH)^{3-}(aq)$ and $Zn(OH)_4^{2-}(aq)$. (Reichle, et al., 1975)

Figure 13 presents the effect of Na_2O and ZnO concentration on the solubility of ZnO in aqueous NaOH at four different temperatures. The solubility of ZnO was increased until a maximum solubility was reached and there after increased NaOH concentration did not improve the solubility of ZnO. The maximum solubility of $NaZn(OH)_3$ increased with temperature. (Chen, et al., 2012)

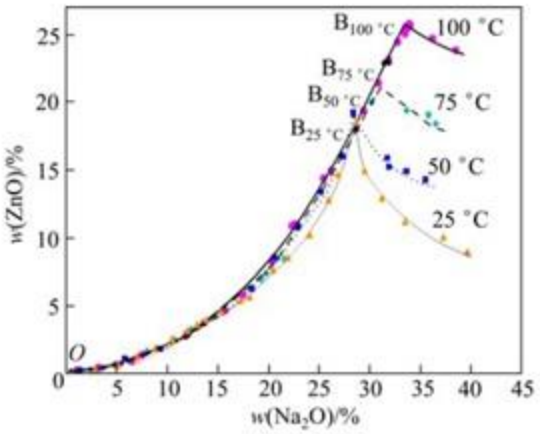

Figure 13 *Solubility of zinc in Na2O/ZnO/H2O system. (Chen, et al., 2012)*

2.4.2 Cellulose phase diagrams

NaOH and cellulose produce a crystalline complex above certain concentrations. This complex holds number of NaOH and water molecules within the crystalline lattice. Different Na-cellulose allomorphs can be obtained by immersing cellulose in various NaOH concentrations. At 12-15% NaOH concentration Na-Cellulose I is produced. At higher NaOH concentration (typically 32%) Na-Cellulose II is produced. This allomorph has two forms: colorless II_A and bright blue II_B. The development of these two forms depends on whether cellulose sample were constrained or not during the transformation. Vacuum drying Na-Cellulose produces Na-Cellulose III. Washing Na-cellulose I and II until neutral produce sodium free Na-Cellulose IV. (Porro, et al., 2007)

Figure 14 presents a phase diagram of Na-cellulose. This phase diagram is based on the occurrence of Na-Cellulose I and II with the Q region. In this Q region the cellulose (e.g. cotton) is basically soluble. In the phase diagram six regions is presented. Two of these regions are border zones, where two phases exists at the same time. At the Q region the increase of the swelling and dissolving power of NaOH towards cellulose at low temperature. (Porro, et al., 2007)

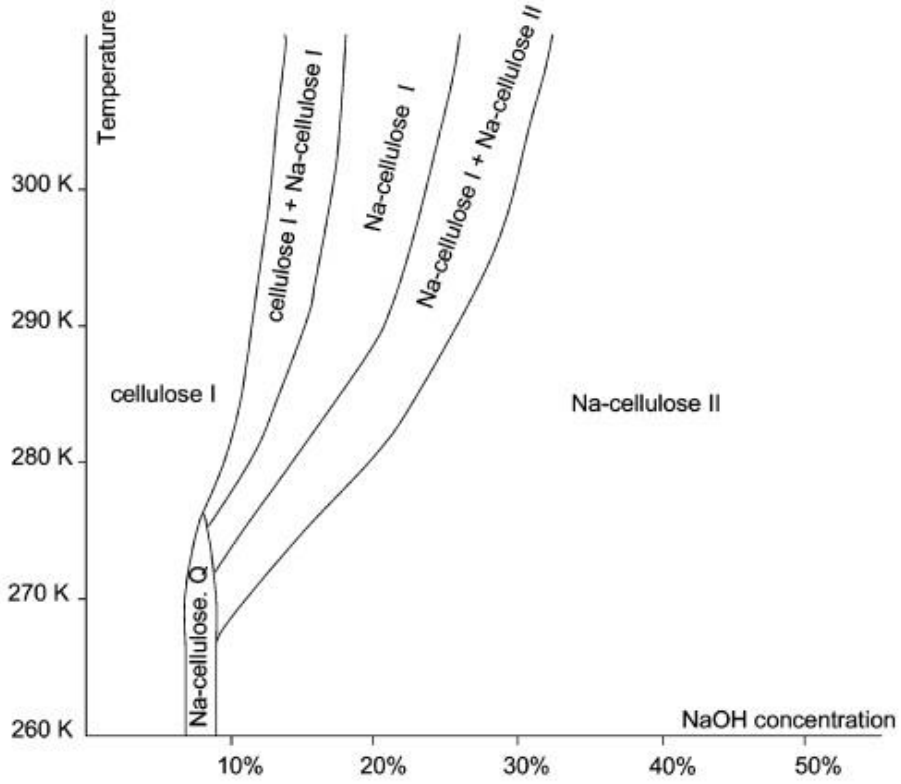

Figure 14 *Phase diagram of Na-cellulose from cotton immersed into aqueous NaOH solutions. (Porro, et al., 2007)*

Cai and Zhang (2005) studied the solubility of cotton linter pulp in NaOH/urea aqueous solution. Figure 15 presents three dimensional phase diagram of solubility of cellulose in NaOH/urea aqueous solution. It can be seen from the diagram that cellulose can be dissolved in the concentration range of 6-10 % of NaOH and 2-20 % of urea at the temperature of -20 to -5 °C. At high concentration of NaOH and urea the solution is unstable. (Cai & Zhang, 2005)

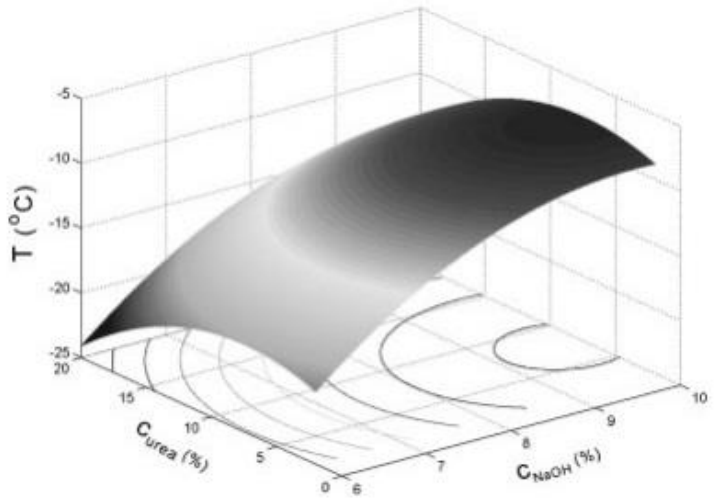

Figure 15 *Phase diagram of cellulose in NaOH/Urea aqueous solution. (Cai & Zhang, 2005)*

N-Methylmorpholine-N-oxide (NMMO) and water mixture is a direct solvent for cellulose. To dissolve cellulose, the amount of water in NMMO is critical: the amount of water has to be less than one molecule per NMMO molecule. In the Lyocell process cellulose is dissolved into NMMO and regenerated with water. (Biganska & Navard, 2003)

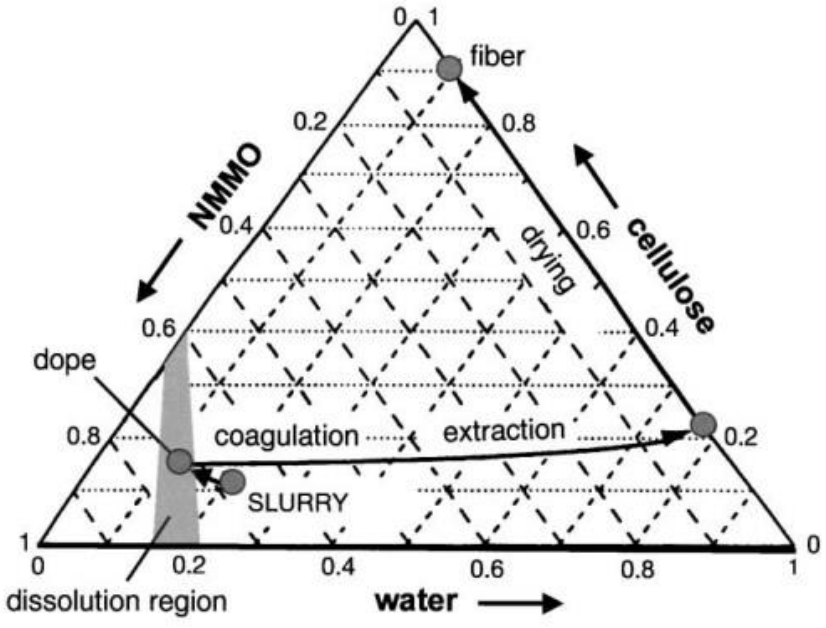

Figure 16 *Phase diagram cellulose/NMMO/water. (Wachsmann, et al, 1997, cited in Fink, et al., 2001)*

Figure 16 presents the solubility of cellulose in binary NMMO-water mixture. It can be seen from this diagram that cellulose dissolves completely in to highly concentrated NMMO in small region. The dissolution process starts with a composition outside this

region. This mixture produces slurry of cellulose and NMMO, where the amount of water is to excess. In this mixture cellulose doesn't dissolve, but in this step is needed to wet the cellulose thoroughly. The excess water is extracted by raising temperature and reducing pressure done until the region of complete dissolution is reached (13-15 wt% water). Here a homogenous and shapeable cellulose solution is formed. (Fink, et al., 2001)

Another solvent group for cellulose is the ionic liquids (IL). They are organic salts that exist as liquids at relatively low temperatures. Ionic liquids are non-volatile, have high dissolving capacity and high thermal stability. Most of the studied ionic liquids as a solvent for cellulose are imidazolium-based ionic liquids, such as 1-ethyl-3-methylimidazolium acetate (EmimAc), 1-butyl-3-methylimidazolium chloride (BmimCl) and i-allyl-3-methylimidazolium chloride (AmimCl). (Le, et al., 2014; Zhu, et al., 2006)

Along with ionic liquids "co-solvents" are present. Most important co-solvent is water, which is used for the coagulation of cellulose and has high hydrophilicity towards imidazolium-based ionic liquids. However adding water disrupts the interaction between cellulose and ionic liquid, and decreases the cellulose solubility. Understating the limits for water content in cellulose-ionic liquid system affects the recyclability of ionic liquid. Second important co-solvent for ionic liquids is dimethylsulfoxide (DMSO). DMSO advances cellulose dissolution in ionic liquids by decreasing the solvent and viscosity of the solution. (Le, et al., 2014)

Figures 17 and 18 present the phase diagrams of cellulose in EmimAc-DMSO and EmimAc-water mixtures. The maximum cellulose concentration ($C_{cell\ max}$) depends on the concentration of DMSO or water in the solution. The higher fraction of DMSO or water in the mixture lowers the maximum amount of cellulose, which is possible to dissolve in the given mixture. DMSO and water have different maximum dissolved cellulose concentration, for example $C_{cell\ max}$ at $wt\%_{DMSO}$=10%, which is five times higher than $C_{cell\ max}$ at the same water concentration. (Le, et al., 2014)

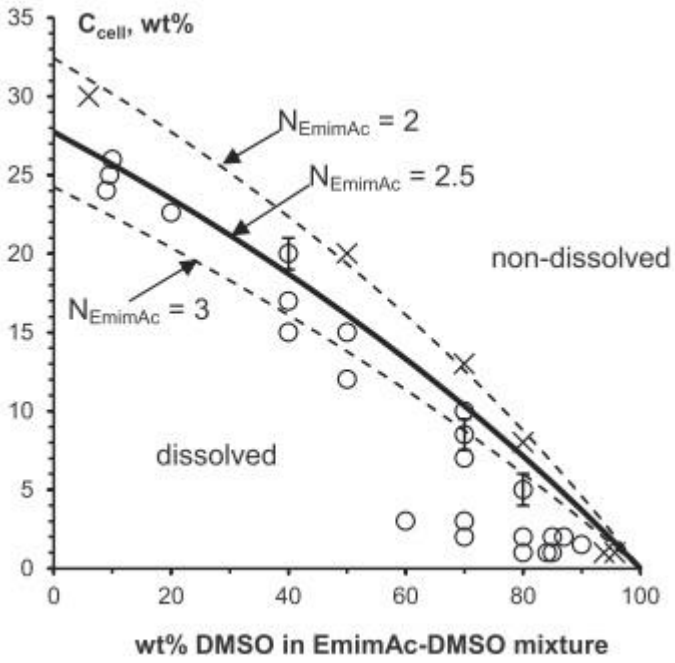

Figure 17 *Phase diagram of cellulose in EmimAc/DMSO mixture. Solid line presents the maximal cellulose concentration calculated supposing no interaction between EmimAc and DMSO and 1 AGU binding N_{EmimAc}=2,5 moles. Dashed lines presents the AGU binding N_{EmimAc}= 3 and 2 moles of EmimAc. Open points presents the transparent and dark solution. Crosses presents the cases with few small non-dissolved particles were detected. (Le, et al., 2014)*

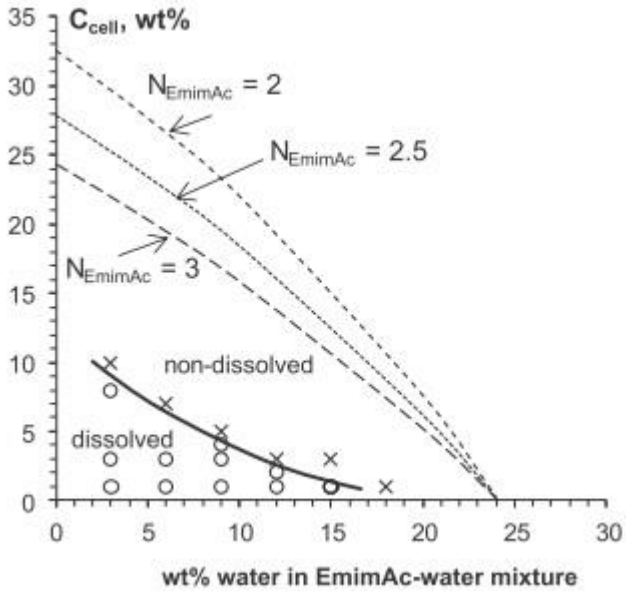

Figure 18 *Phase diagram of cellulose in EmimAc/water mixture. Solid line presents the approximate maximal limit of cellulose concentration. Dashed lines corresponds the solid and dashed lines from figure 16. (Le, et al., 2014)*

Figure 19 presents the ternary phase diagram for cellulose/EimimAc/DMSO and cellulose/EimimAc/water solutions. The region, where cellulose is soluble, is shaded in the diagram. (Le, et al., 2014)

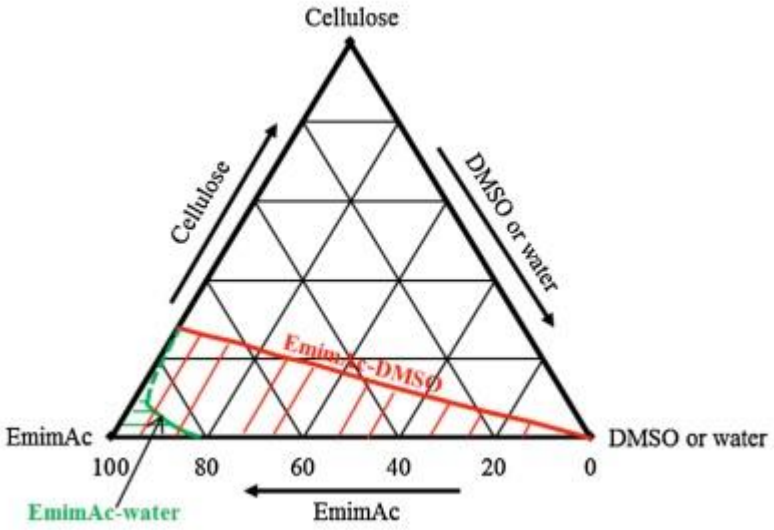

Figure 19 *Ternary phase diagram for cellulose/EimimAc/DMSO and cellulose/EimimAc/water solutions. Concentrations are in wt%. (Le, et al., 2014)*

3. MATERIALS AND METHODS

3.1 Enzyme-treated pulps

The starting pulps of the studies were low and medium viscosity dissolving grade pulp produced by Domsjö Fabriker AB (Örnsköldsvik, Sweden). The pulp was wetted and mechanically shredded for 5 hours using Baker Perkins shredding machine as in Vehviläinen et al. (2015). After the mechanical shredding the pulp was treated with a commercial enzyme preparation, Biotouch C39 (AB Enzymes Oy, Finland) at 5% cellulose consistency at pH6, 60 °C for 3 hours. The enzyme dosage was 1 mg protein/g oven dry pulp. This low viscosity grade pulp is referred as Pulp1.

The medium viscosity pulp was treated with a laboratory scale twin screw extruder at VTT as described in Grönqvist et al. (2015). The pulp was passed through the extruded twice: the first pass without enzymes and the second pass with enzymes. The enzyme was Fibercare R (Novozym) with the dosage of 0.25 mg/g. This medium viscosity grade pulp is referred as Pulp2.

The soluble sugars during the enzyme treatment were measured with 3,5-dinitrosalisylic acid (DNS) method as described in Vehviläinen et al. (2015). The intrinsic viscosity before and after the enzyme treatment was measured according to the SCAN-CM 15:99. The characteristics of pulps are given in Table 1.

Table 1 *Characterization of enzyme treated pulp. Pulp1 was measured at TUT and Pulp2 at VTT.*

Characterization	Pulp1	Pulp2	Test method
Soluble sugars during enzyme treatment mg/g	19	10	DSN
Viscosity of the pulp ml/g **Before the enzyme treatment** **After the enzyme treatment**	 430 233	 550 210	SCAN-CM 15:99

3.2 Experiments

3.2.1 Preparation of solvents

The stock solutions (concentrated sodium zincate solutions) were prepared as follows: solid sodium hydroxide and solid zinc oxide were weighted into a beaker (Table 2) and

water was added. Water was added to these stock solutions (Table 3). The density solutions 2, 3 and 5 solutions were determined to have the right composition of the solvent (Table 3). Zinc oxide did not dissolve in the solution 4.

Table 2 *The composition of the stock solutions presented as percentage by mass.*

Solution	NaOH wt%	ZnO wt%
1	35	7
2	20	16,3
3	20	40,7
4	20	65,1
5	20	52,9

Density of the solvents

The density of the selected solvents was measured as follows. A Beaker was tared. 2 ml of each solution is pipetted to the breaker and the weight is written down. Another 2 ml is added without resetting the scales and the weight is written down. These additions are done 10 times altogether to have a volume of 20 ml.

A graph is drawn from the measurement data. The volume is presented in x axis and the weight is in y axis. The density is calculated by using the slope of the graph:

$$k = \frac{\Delta y}{\Delta x}$$

Table 3 *Characteristics of the samples.*

Sample	Stock Solution	Cellulose (wt%)	NaOH (wt%)	ZnO (wt%)
1	1	1	1,08	0,2
2	1	3,5	3,8	0,8
3	1	4,5	4,9	1
4	1	5	5,4	1,1
5	1	5,5	6	1,2
6	1	6	6,5	1,3
7	1	6,5	7	1,4
8	1	7	7,6	1,5
9		6	6,5	0
10		6	6,5	1
11		1	6,5	0
12	1	1	6,5	1,3
13	2	4	4	0,3
14	2	4	5,2	0,4
15	2	4	6,4	0,5
16	2	4	9,6	0,8
17	3	4	4	0,8
18	3	4	5,2	1,1
19	3	4	6,4	1,3
20	3	4	9,6	2,0
21	5	4	4	1,1
22	5	4	5,2	1,4
23	5	4	6,4	1,7
24	5	4	9,6	2,5

3.2.2 Solubility

The solubility was measured by preparing 20 g of alkaline cellulose sample. The pulp was weighted and water was added to adjust the dry content to 15%. The stock solution was diluted with water and added to the pulp. The sample was mixed at room temperature for 5 minutes. Then the sample was placed at desired temperature at least for 18 hours (Table 3).

Table 4 *The Temperatures of the experiments.*

Temperature (°C)
-40
-15
15
20

Subzero treatment

After 18 hours at desired subzero temperature the sample is placed in glycol bath at the temperature of 15 °C and let to melt. After the solution had melted, it was diluted with 6.5% or 4% NaOH and the insoluble part was separated from the solute part in a centrifuge (3000 g, 15 min). If the insoluble part had enough cohesion it was dried on glass plate, precipitated in 10% H_2SO_4 and washed with water. Otherwise the insoluble part was washed with dialysis. The pure insoluble cellulose was dried overnight in an oven (105 °C) before weighing. The solubility percentage is calculated by following equation (1):

$$Solubility\ \% = \frac{m(pulp) - m(insoluble)}{m(pulp)} * 100\% \quad (1)$$

Treatment above zero

After 18 hours at desired temperature above zero the sample was diluted with 6.5% or 4% NaOH and the insoluble part was separated from the solute part in a centrifuge (3000 g, 15 min). The insoluble part was washed with dialysis until neutral. The pure insoluble cellulose was dried overnight in an oven (105 °C) before weighing it. The solubility percentage was calculated according to equation (1).

The insoluble part was studied with an optical microscope (Leitz Laborlux D).

4. RESULTS AND DISCUSSION

4.1 The Effect of ZnO on solubility

The effect of ZnO on solubility of Pulp1 was studied with the constant NaOH concentration (6.5 wt%). The cellulose concentration was 1 wt% and 6 wt%. ZnO concentration was varied at the range on 0-1.3 wt%. The samples were frozen at -40 °C.

The effect of ZnO on solubility is presented in Figure 20. It was clearly seen that ZnO had an effect on the solubility of cellulose in NaOH solution positively. With 1.0 wt% of cellulose (light columns in Figure 20) the insoluble part was impossible to extract due to the very small fraction. However, according to the visual examination the solution with ZnO was clear, whereas the solution without ZnO was cloudy indicating the difference in solubility. With the higher cellulose concentrations (6.0 wt%) it was possible to detect difference in solubility. The solubility increases as the concentration of ZnO increases (dark columns in Figure 20). The solubility was 53% at the without ZnO and increased to 93% with the addition of ZnO (1.3 wt%). These results are in line with studies of Yang, et al. (2010), Yang, et al. (2011) and Vehviläinen, et al. (2015), who observed that the solubility of cellulose in NaOH and NaOH/urea was enhanced by the addition of ZnO. Yang et al (2011) suggested that the zincate ion formed stronger hydrogen bonds between cellulose than NaOH hydrate.

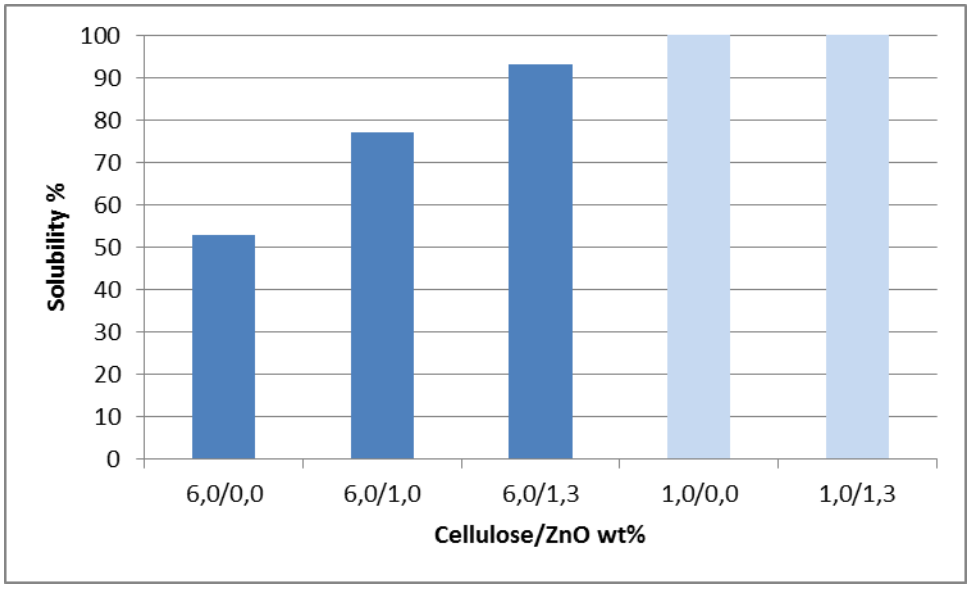

Figure 20 *The effect of ZnO in solubility. The concentration of NaOH was 6.5 wt% for all solutions.*

4.2 Solubility with constant AGU:NaOH ratio

Based on the earlier studies by Vehviläinen, et al. (2015) the best solution in the freeze-thaw method was obtained with following concentrations: 6 wt% cellulose, 6.5 wt% NaOH and 1.3 wt% ZnO. From this solution the following mole ratios were calculated:

1. AGU:NaOH (0.023)
2. ZnO:NaOH (0.098)

The molar mass of one AGU is 162 g/mol. The mole ratios were held constant and the concentration of cellulose was changed during the solubility trials with the Pulp1. Due to the changes in cellulose concentration the concentrations of NaOH and ZnO changed.

The effect of cellulose concentration on the solubility of Pulp1 at constant ratio between AGU and NaOH is presented in Figure 21. Since the molar ratio was kept constant by changing the concentration of cellulose, the content of other components of the solution was changed as well. At the concentration of 1 wt% of cellulose, the concentration of NaOH was 1.08 wt% and ZnO 0.22 wt%. With this low concentration, NaOH was unable to dissolve cellulose despite of low cellulose concentration. According to Egal (2006) and Zhang, et al. (2011) with thew low NaOH concentration, the NaOH-water hydrates were too large to penetrate into the crystalline region of cellulose.

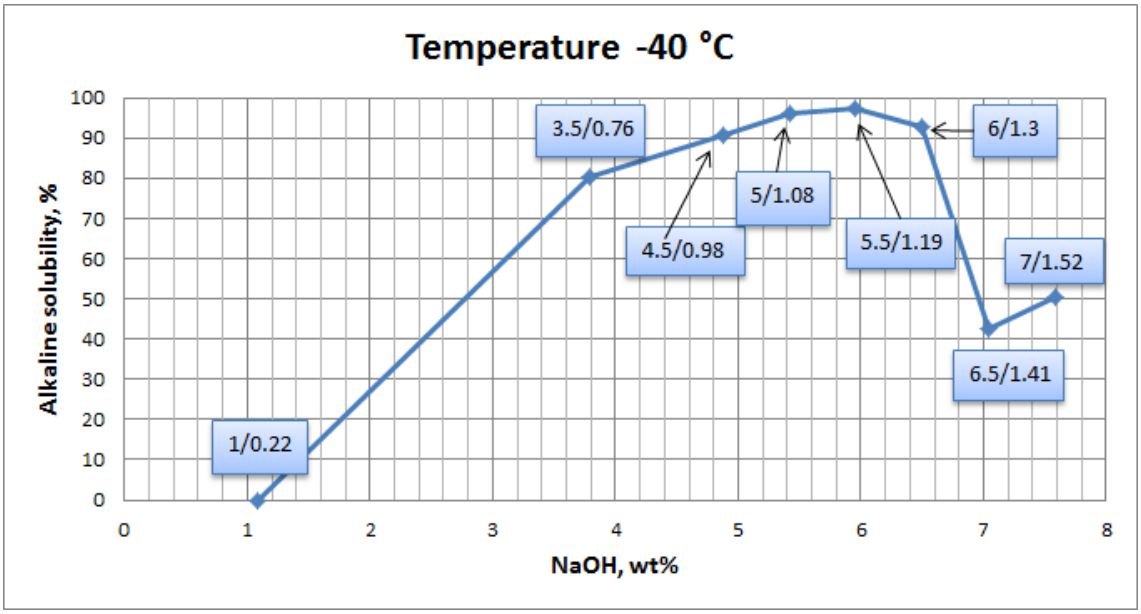

Figure 21 *Effect of NaOH concentration on solubility of cellulose at -40 °C. Also the concentration of cellulose/ZnO (wt%) of each sample is presented.*

In these studies the best solubility was obtained when the cellulose concentration was between 5-6 wt%, NaOH concentration between 5.4-6.5 wt% and ZnO concentration between 1.08-1.3 wt%. This is in line with the studies of Vehviläinen, et al. (2015) where cellulose was dissolved with the similar methods as used here. They found that

the most thermally stable solution had 5.9 wt% cellulose, 6.5% NaOH and 1.3 wt% ZnO. Ciechańska, et al. (2005) found an optimal solution containing 6 wt% cellulose 7.8 wt% NaOH and 0.87 wt% ZnO. In this study the pretreatment of the pulp was the same, but the dissolution method was different. They used higher temperature and mixing was involved.

At the higher concentration (6.5 wt% cellulose/7.04 wt% NaOH/1.41 wt% ZnO and 7 wt% cellulose/7.59 wt% NaOH/ 1.52 ZnO) NaOH dissolved more cellulose at room temperature which led to the formation of gel. Similar results have been obtained by Vehviläinen, et al. (2015) with cellulose/NaOH/ZnO solution and Cai & Zhang (2005) with cellulose/NaOH/urea solutions.

Figure 22 presents the microscopic images of insoluble parts of selected solutions. The insoluble fraction of the solution with 6 wt% cellulose had swollen fiber fragments (Figure 22 a) The insoluble part of the solution with 6.5 wt% cellulose had swollen fiber fragments and gelled network-like pattern (Figure 21 b).

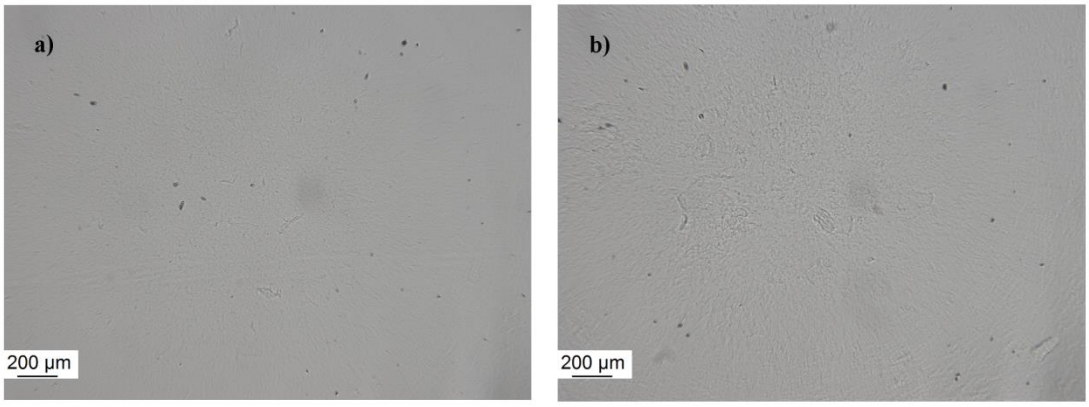

Figure 22 *Optical microscope images of insoluble parts of solution with a) 6 wt% cellulose/6.5 wt% NaOH/1.3 wt% ZnO and b) 6.5 wt% cellulose/7.04 wt% NaOH/1.41 wt% ZnO.*

The decreased solubility at the concentration of 6.5 wt% NaOH indicated the dissolution of cellulose already during the mixing step at the room temperature. To study this further the solubility of selected solutions was studied right after mixing at 20 °C (Figure 23). With low NaOH concentrations the solubility of cellulose was low (under 10%). With 5.96 wt% NaOH the solubility increased to 12% and raised over 30% with NaOH concentrations over 7 wt%. If cellulose dissolves before freezing step, it causes gel forming and leads to decreased solubility.

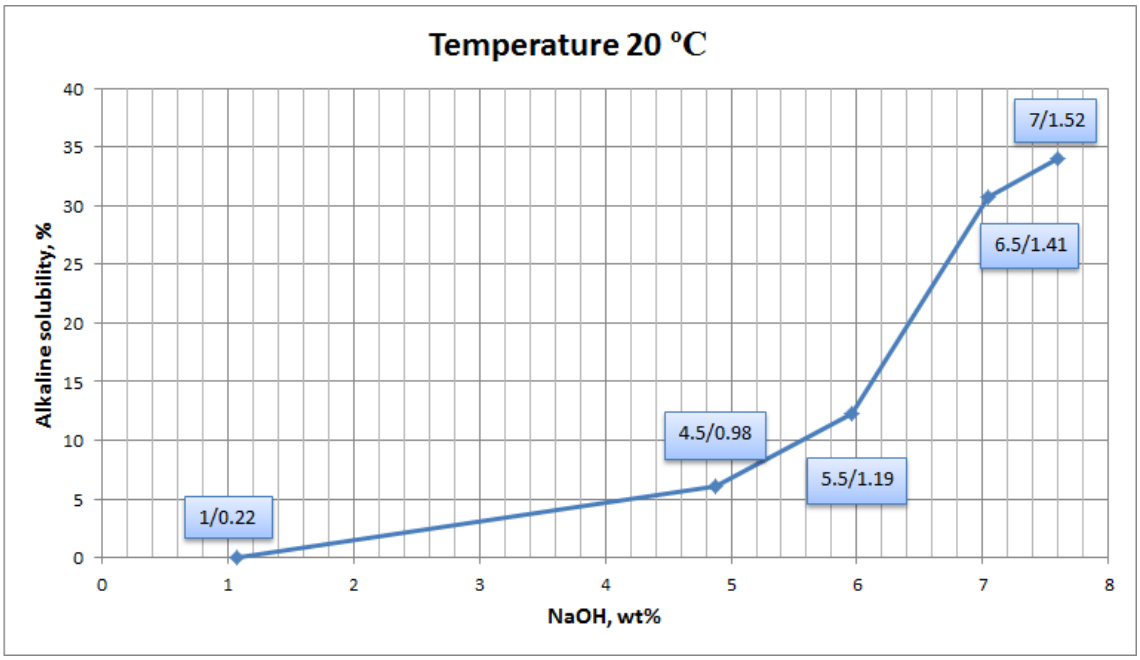

Figure 23 *Effect of NaOH on solubility of cellulose at 20 °C. Also the concentration of cellulose/ZnO (wt%) of each sample is presented.*

4.3 Effect of NaOH and ZnO concentrations on solubility

The solubility of Pulp2 was studied at constant cellulose concentrations 4 wt% at two temperatures -15 °C and 15 °C. The solubility is examined in three different ZnO and NaOH molar ratio:

1. 0.04
2. 0.10
3. 0.13

and four different NaOH concentrations:

1. 1.0 mol (4 wt%)
2. 1.3 mol (5.2 wt%)
3. 1.6 mol (6.4 wt%)
4. 2.4 mol (9.6 wt%)

Figure 24 presents the result of the solubility determination at -15 °C. Clearly all the studied ZnO/NaOH molar ratios, the solubility increased with the increase of NaOH concentration. At the lowest molar ratio (0.04) the solubility was at the lowest compared to other molar ratios despite of the NaOH concentration. To have solubility over 95% the concentration of NaOH was 1.6 mol at the lowest ZnO/NaOH molar ratio. On the other molar ratios NaOH concentration of 1.3 mol was enough to achieve solubility over 95%.

Figure 25 presents the optical microscopic images of the insoluble parts. Fibre fragments were not found from the insoluble parts of the samples with highest solubility (over 98%). The images in Figure 25 correlate with the solubility, where the cellulose fibre swelled and dissolved more with the increase of the concentration of NaOH and ZnO. At low concentrations the insoluble part consisted of whole swollen fibre. As the NaOH concentration raised the insoluble part consist of only swollen fibre fragments. The ballooning effect wasn't present, which indicates that the fibres dissolved first by swelling and then fragmenting and it is not depended of the concentration of NaOH and ZnO.

NaOH concentration between 6 and 10 wt% has been reported to dissolve cellulose (Cai & Zhang, 2005; Egal, 2006; Isogai & Atalla, 1998). Here at higher concentration of NaOH (6.4-9.6 wt%), the solubility of cellulose was over 90% regardless of the amount of ZnO. The increased concentration of ZnO resulted better dissolution of cellulose even at lower concentration of NaOH (5.2 wt%).

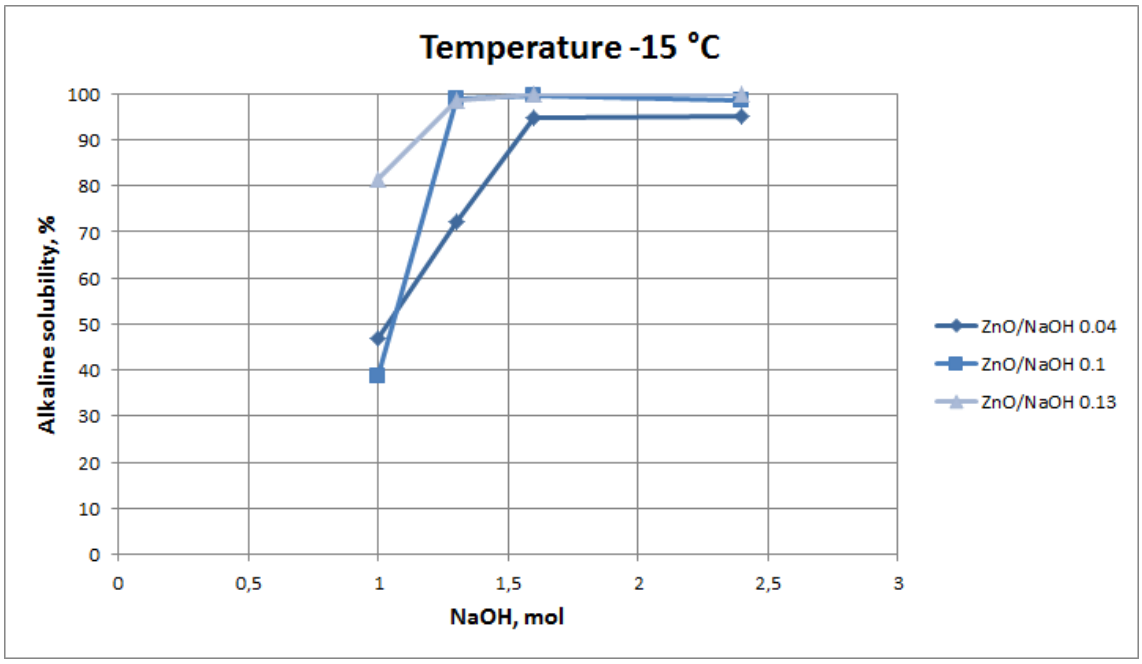

Figure 24 *Effect of NaOH concentration (in moles) on solubility at -15 °C. The concentration of cellulose was 4 wt% for all samples.*

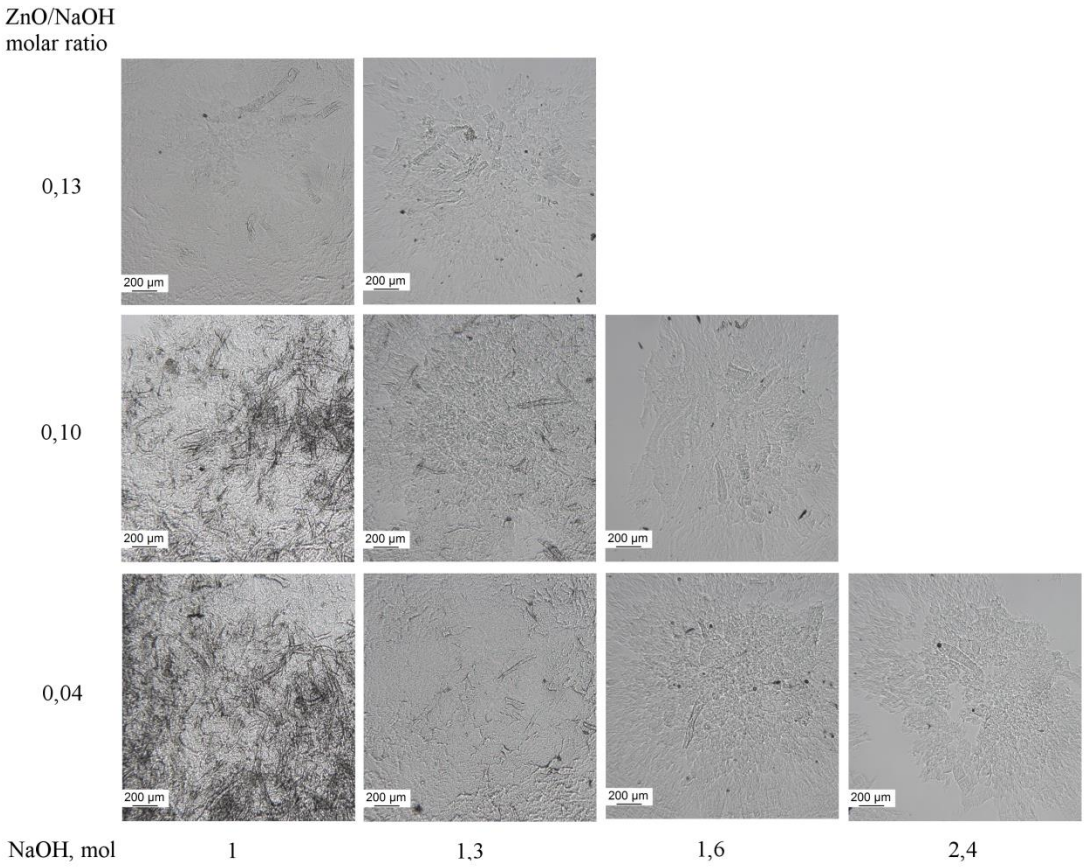

Figure 25 *Optical microscope images of the insoluble parts of solutions dissolved at -15 °C. The concentration of NaOH is presented horizontally and the molar ratio of ZnO/NaOH is presented vertically.*

Figure 26 presents the result of the solubility determination at 15 °C. With 2.4 mol NaOH, cellulose dissolved during the mixing of the pulp and solvent. In the other samples the pulp was clearly still in fibre form before placing them in glycol bath.

Figure 27 presents the optical microscopic images of the insoluble parts of the solutions. At the highest concentration NaOH (2.4 mol) was able to swell the insoluble fibre. On the other concentrations NaOH dissolved only the most accessible parts of the fibres leaving most of them at nearly original fibre forms and no swelled fibres were detect.

Also here increased NaOH concentration increased the solubility of cellulose. At high concentration of NaOH (2.4 mol) the dissolution of cellulose was at the highest. However ZnO didn't seem to have as big effect on the dissolution as it had on low temperature. With the lower NaOH concentrations adding ZnO seemed to decrease the solubility as the concentration of ZnO increased. According to Yang, et al. (2010) overfull ZnO led to the decreased contribution of NaOH and decreased solubility. According to Liu, et al. (2011) in dissolved state ZnO forms hydrates and therefore ZnO acts as a free water binder. Since the temperature in our studies was 15 °C there was more free water presented unlike in lower temperature where water was partially frozen

and zinc might form the hydrates with water instead of bonding with cellulose. NaOH seemed to be the main dissolving agent at this temperature. At the highest NaOH concentration the amount of NaOH was high enough to dissolve cellulose significantly more. The addition of ZnO seemed to increase solubility, but there wasn't a significant difference at the highest ratio (0.1 and 0.13).

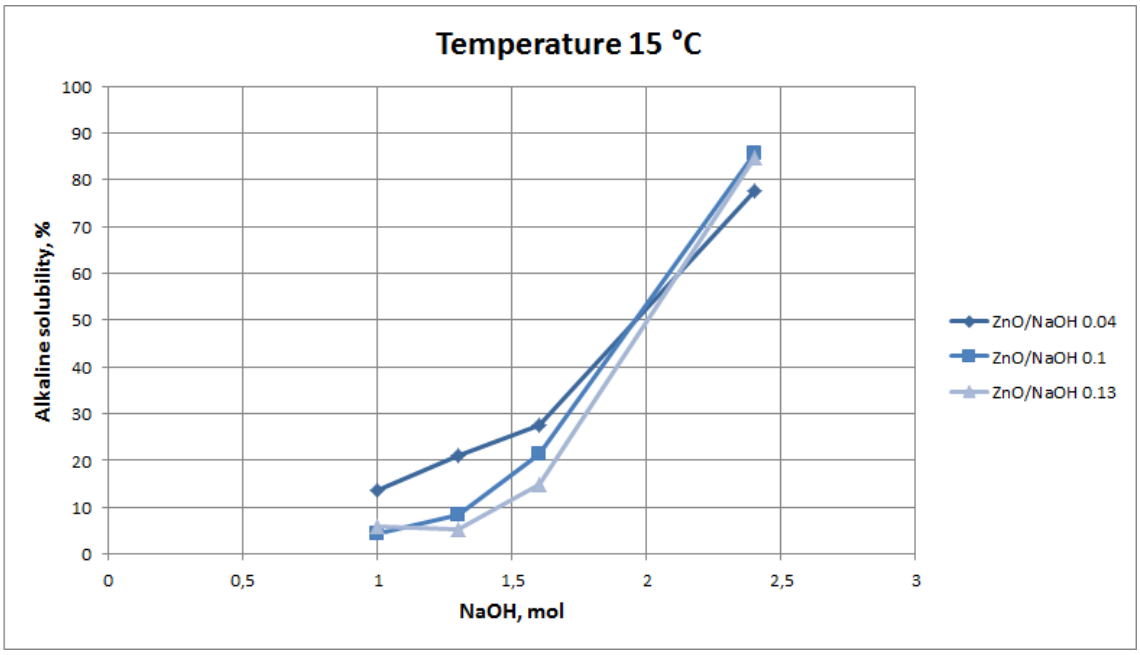

Figure 26 *Effect of NaOH concentration (in moles) on solubility at 15 °C. The concentration of cellulose was 4 wt% for all samples.*

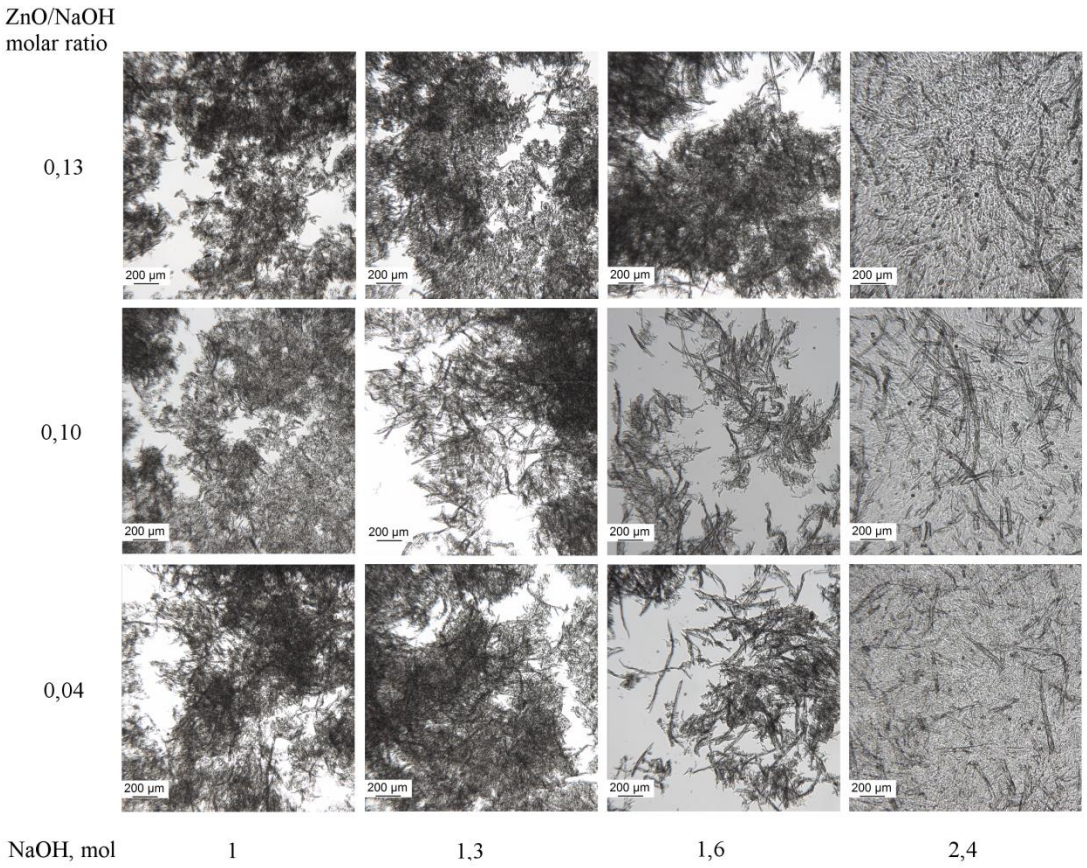

Figure 27 *Optical microscope images of the insoluble parts of the solutions dissolved at 15 °C. The concentration of NaOH is presented horizontally and the molar ratio of ZnO/NaOH is presented vertically.*

5. CONCLUSIONS

There were three different objectives for this thesis. First the effect of ZnO on solubility of cellulose in alkaline solution was studied. Second the solubility of cellulose in alkaline solubility at constant AGU:NaOH molar ratio was studied. Finally the effect of NaOH and ZnO concentrations on solubility of cellulose was studied. The determination of solubility was done by freeze-thaw method. First the cellulose and solvent containing NaOH and ZnO was mixed and then placed at desired temperature. Then se samples were melted and the solubility was determined.

The effect of ZnO on the dissolution of cellulose was clear at low temperature. The addition of small amount of ZnO in the solution, the solubility increased significantly. Also the quality of the solution was affected by ZnO. Comparing low cellulose concentration solution with and without ZnO the solution with ZnO was clear where as the solution without ZnO was cloudy.

At the constant molar ratio between AGU:NaOH and NaOH:ZnO, the best solubility was obtained when the cellulose concentration was between 5-6 wt%, NaOH concentration between 5.4-6.5 wt% and ZnO concentration between 1.08-1.3 wt%. Also it was found, that at very low concentration of cellulose (1 wt%), NaOH wasn't able to dissolve it, due to the low concentration of the reagents. At higher concentration of cellulose (>6.5 wt%) the quality of the solution started to deteriorate. This was probably due to gelation during freezing step. At the concentration of 6.5 wt% NaOH the dissolution of cellulose dropped. This can be explained by the dissolution of cellulose during the mixing step at the room temperature. This was further studied by measuring the solubility of cellulose at 20 °C. The solubility increased clearly with NaOH concentrations above 7 wt%.

In addition, the effect of NaOH and ZnO concentration on the solubility of cellulose was studied at lower cellulose concentrations (4 wt%). It was clear that the solubility increased with the increase of NaOH and ZnO concentrations at low temperature (-15 °C). With the highest studied NaOH concentration (2.4 mol), cellulose already dissolved during the mixing step. With the other studied concentrations of NaOH (1.0 mol, 1.3 mol and 1.6 mol) the pulp was clearly still in fibre form after the mixing. With the lowest studied ZnO/NaOH molar ratio (0.04), the solubility was in each case lower than with the higher ZnO/NaOH molar ratios. With the higher ZnO/NaOH molar ratios (0.1 and 0.13), the solubility was over 95% with NaOH concentrations above 1 mol and the increased NaOH concentration did not have significant effect on the solubility. The solubility was also studied at 15 °C. The solubility of cellulose at the

highest NaOH concentration (2.4 mol) was about 80% and almost three times higher than with the other studied NaOH concentration. The effect of ZnO was insignificant.

Because the solubility at higher concentration of NaOH and ZnO was the same, further study is required. The further studies are suggested including studies about the effect of increased cellulose concentration on the solubility. In addition, further studies with different dissolution temperature may give interesting information about the solubility of cellulose in aqueous NaOH. The challenge is to find the conditions where cellulose is soluble at higher temperature to advance the industrial applications of the studied dissolution method for the regeneration of cellulose fibres.

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APPENDIX A: PARAMETERS FOR SOLUBILITY DETERMINATION

Constant AGU:NaOH ratio.

Sample	1	2	3	4	5	6	7
Pulp (g)	3,7 (1,3)	4,0 (1,4)	0,6 (0,2)	3,1 (1,1)	2,8 (1,0)	2,5 (0,9)	2,0 (0,7)
Pulp, adjusted weight (g)	8,7	9,3	1,3	7,3	6,7	6	4,7
Stock solution (g)	4	4,3	0,6	3,4	3,1	2,8	2,2
Added water (g)	7,3	6,3	28,1	9,3	10,2	11,2	13,2
Diluted stock solution (g)	11,3	10,7	18,7	12,7	13,3	14	15,3

The effect of ZnO on solubility.

Sample	8	9	10	11
Concentrations (pulp/NaOH/ZnO)	6/6,5/0	6/6,5/1	1/6,5/1,3	1/6,5/0
pulp g	3,4 (1,2)	3,4 (1,2)	0,6 (0,2)	0,6 (0,2)
Pulp, adjusted weigth g	8	8	1,3	1,3
Stock solvent	10,8 wt% NaOH	35 wt% NaOH/ 5,38 wt% ZnO	35 wt% NaOH/ 7% ZnO	7 wt% NaOH
stock solvent g	12	3,7	3,7	18,7
added water g	0	8,3	3,7	0
diluted stock solvent g	12	12	18,7	18,7

APPENDIX B: DENSITY DETERMINATION

Stock Solution 1: 20 wt% NaOH, 1.63 wt% ZnO.

Stock Solution	H-12-VL1		H-13-VL1		H-14-VL1		H-15-VL1	
	V (ml)	m (g)	V (ml)	m (g)	V (ml)	m (g)	V (ml)	m (g)
	5	5,27	2	2,13	2	2,09	2	2,16
	10	10,53	4	4,25	4	4,21	4	4,38
	15	15,76	6	6,37	6	6,31	6	6,58
	20	21,05	8	8,47	8	8,33	8	8,8
	25	26,32	10	10,56	10	10,42	10	10,96
	30	31,59	12	12,7	12	12,5	12	13,2
	35	36,82	14	14,77	14	14,56	14	15,4
	40	42,07	16	16,87	16	16,61	16	17,53
	45	47,32	18	18,94	18	18,74	18	19,67
			20	20,9	20	20,84	20	21,75
Slope		1,05		1,05		1,04		1,09

Stock Solution 2: 20 wt% NaOH, 4.02 wt% ZnO.

Stock Solution	H-16-VL2		H-17-VL2		H-18-VL2		H-19-VL2	
	V (ml)	m (g)	V (ml)	m (g)	V (ml)	m (g)	V (ml)	m (g)
	2	2,14	2	2,05	2	2,17	2	2,17
	4	4,21	4	4,11	4	4,34	4	4,37
	6	6,32	6	6,17	6	6,51	6	6,56
	8	8,4	8	8,23	8	8,67	8	8,76
	10	10,47	10	10,28	10	10,84	10	10,98
	12	12,55	12	12,28	12	13,02	12	13,18
	14	14,61	14	14,37	14	15,17	14	15,39
	16	16,69	16	16,44	16	17,33	16	17,58
	18	18,79	18	18,5	18	19,47	18	19,79
	20	20,86	20	20,51	20	21,6	20	21,97
Slope		1,04		1,03		1,08		1,10

Stock Solution 3: 20 wt% NaOH, 5.29 wt% ZnO.

Stock Solution	H-20-VL3		H-21-VL3		H-22-VL3		H-23-VL3	
	V (ml)	m (g)	v (ml)	m (g)	V (ml)	m (g)	V (ml)	m (g)
	2	2,15	2	2,16	2	2,2	2	2,21
	4	4,27	4	4,32	4	4,38	4	4,44
	6	6,39	6	6,48	6	6,55	6	6,58
	8	8,51	8	8,63	8	8,72	8	8,84
	10	10,64	10	10,79	10	10,9	10	11,07
	12	12,77	12	12,95	12	13,08	12	13,26
	14	14,9	14	15,11	14	15,25	14	15,48
	16	17,02	16	17,28	16	17,43	16	17,73
	18	19,15	18	19,43	18	19,62	18	19,95
	20	21,26	20	21,58	20	21,81	20	22,15
Slope		1,06		1,08		1,09		1,11

APPENDIX C: PARAMETERS AND RESULTS FOR THE EFFECT OF NAOH AND ZNO CONCENTRATIONS ON SOLUBILITY

The parameters for the experiment

Sample	NaOH (mol)	NaOH (g/l)	NaOH (wt%)	ZnO/NaOH molar ratio	ZnO (mol)	ZnO (g/l)	ZnO (wt)%
12	1	40,01	4	0,04	0,04	3,26	0,326
13	1,3	52,01	5,2	0,04	0,05	4,2	0,42
14	1,6	64,02	6,4	0,04	0,06	5,2	0,52
15	2,4	96,02	9,6	0,04	0,1	7,8	0,78
16	1	40,01	4,0	0,1	0,1	8,1	0,81
17	1,3	52,01	5,2	0,1	0,13	10,58	1,06
18	1,6	64,02	6,4	0,1	0,16	13,02	1,3
19	2,4	96,02	9,6	0,1	0,24	19,53	1,95
20	1	40,01	4	0,13	0,13	10,58	1,06
21	1,3	52,01	5,2	0,13	0,17	13,75	1,38
22	1,6	64,02	6,4	0,13	0,21	16,93	1,69
23	2,4	96,02	9,6	0,13	0,31	25,39	2,54

Diluting of stock solution. The weight of the final solution was 19.2 g.

Sample	Diluted Stock Solution (ml)	NaOH (g)	Stock Solution (ml)	Water (ml)	Density of dilut-ed stock solution (g/ml)	Wight of diluted solution (g)	Water present in cellu-lose 85% (g)
12	18,26	0,73	3,65	14,61	1,05	19,2	4,53
13	18,35	0,95	4,77	13,58	1,05	19,2	4,53
14	18,49	1,18	5,92	12,57	1,04	19,2	4,53
15	17,59	1,69	8,45	9,14	1,09	19,2	4,53
16	18,46	0,74	3,69	14,77	1,04	19,2	4,53
17	18,71	0,97	4,87	13,84	1,03	19,2	4,53
18	17,78	1,14	5,69	12,09	1,08	19,2	4,53
19	17,44	1,67	8,37	9,067	1,1	19,2	4,53
20	18,07	0,72	3,61	14,46	1,06	19,2	4,53
21	17,79	0,93	4,63	13,16	1,08	19,2	4,53
22	17,63	1,13	5,64	11,99	1,09	19,2	4,53
23	17,32	1,66	8,32	9	1,11	19,2	4,53

Test Series A, temperature -15 °C.

Sample	Pulp (g)	Dry Content %	Weight Pulp + Water (g)	Insoluble Part (g)	Solubility %
12-A	4,24	18,88	0,8	0,42	47
13-A	4,24	18,88	0,8	0,22	72
14-A	4,24	18,88	0,8	0,04	95
15-A	4,24	18,88	0,8	0,04	95
16-A	4,24	18,88	0,8	0,49	39
17-A	4,24	18,88	0,8	0,01	99
18-A	4,24	18,88	0,8	0,003	100
19-A	4,24	18,88	0,8	0,01	99
20-A	4,24	18,88	0,8	0,15	8
21-A	4,24	18,88	0,8	0,01	99
22-A	4,24	18,88	0,8	0	100
23-A	4,24	18,88	0,8	0	100

Test Series B, temperature 15 °C.

Sample	Pulp (g)	Dry Content %	Weight Pulp + Water (g)	Insoluble Part (g)	Solubility %
12-B	4,24	18,88	0,8	0,69	14
13-B	4,24	18,88	0,8	0,63	21
14-B	4,24	18,88	0,8	0,58	27
15-B	4,24	18,88	0,8	0,18	78
16-B	4,24	18,88	0,8	0,77	4
17-B	4,24	18,88	0,8	0,73	8
18-B	4,24	18,88	0,8	0,63	21
19-B	4,24	18,88	0,8	0,11	86
20-B	4,24	18,88	0,8	0,75	6
21-B	4,24	18,88	0,8	0,75	5
22-B	4,24	18,88	0,8	0,68	15
23-B	4,24	18,88	0,8	0,12	85